THE JOURNAL OF
PEDIATRICS

SEPTEMBER 1960 *Volume* 57 *Number* 3

# *Lawson Wilkins*

LAWSON WILKINS graduated from the Johns Hopkins Medical School in 1918, close to if not actually at the head of his class, but received his degree *in absentia* while in France as a member of the Johns Hopkins Hospital Unit in World War I. The Johns Hopkins medical students who had volunteered were given jobs as orderlies. One day they were summoned by the Adjutant, who informed them that they had just been accorded M.D.'s by the University and that they were now physicians. Having made this brief announcement he remarked, "Now go back to the work you are doing." At various times Lawson has described to me his experiences as an orderly and how expert he became with the bedpan.

When, at the expiration of the war, Lawson returned to this country and obtained his discharge from the army, he discovered that the internships at the Johns Hopkins Hospital had already been assigned. However, he obtained an internship in internal medicine at the New Haven Hospital under two of the ablest internists in the United States, Dr. George Blumer, a former pupil of Dr. Osler, and Dr. Wilder Tileston.

When Lawson had finished his year at New Haven, he determined to go into pediatrics and accepted an internship at the Harriet Lane Home. The new pediatrics under Dr. Howland's guidance was then rising into its fullness and his clinic at Johns Hopkins had become the outstanding success which ensured the spread of the "full-time" movement. I had always supposed that Lawson, like Wilburt Davison and others, was inveigled into pediatrics by Dr. Howland's powerful and magnetic personality, but he has said to me that he chose it because he thought it was the branch of medicine then leading in the biochemical and metabolic approach. Dr. Howland's influence was, therefore, indirectly the basis for his choice.

It is worth noting that while an intern Lawson started the special clinic for congenital syphilis. He was impressed by the poor care which these unfortunate children were receiving in the outpatient department, assigned as they were in haphazard fashion to a staff of interns untrained in the knowledge of the disease and in technical methods required; moreover, there was no follow-up system. Lawson organized their care and obtained a special social worker. The outcome was Dr. Howland's authorization of a special

clinic for congenital syphilis. This was the only special clinic in existence until 1927.

At the close of his internship at the Harriet Lane Home, Lawson decided in favor of a life devoted to private practice. He made this decision as the result of his father's hold upon him. I never had the privilege of meeting Lawson's father but he must have been a remarkable man. He was a general practitioner in Baltimore who continued his work literally to the time of his death at 86 years, in the last few days bringing his patients to his bedside. "If the child is the father of the man," Dr. Wilkins, Sr., must have been much like the son, tireless, alert to progress, open-minded to new ideas. Lawson has told me that his father was probably the first physician in Baltimore to employ the clinical thermometer and to use diphtheria antitoxin. Lawson's mother had died when he was 5 years old and the relations with his father were always exceedingly close. The elder Wilkins had always had an intense interest in his fellow human beings and their individual problems and could not conceive of complete satisfaction in life apart from the intimate human contact which practice afforded. It was the acceptance of the father's feeling for what was most worth while in life which caused the son to decline Dr. Howland's offer of an assistant residency which was at the time the open gateway to an academic career.

Lawson practiced pediatrics for the ensuing 25 years. I believe that he had the largest pediatric practice in Baltimore. It might be termed an eminently democratic practice, for it was not confined to the financially "top drawer" because by nature Lawson responded to the human need wherever it happened to be and the financial reward was not the consideration. Moreover, his fees were said always to be moderate. The burden of work which he carried was tremendous and I happen to know that he often did not return home until 8, 9, or 10 in the evening and, no matter how delayed, he always found his faithful wife, Lucille, patiently waiting. With a complete generosity she adapted her life entirely to helping him in every possible way and he reciprocated with an intense devotion.

I cannot recall when my intimate knowledge of Lawson first began. He was a student when Dr. Howland delegated me to teach nutrition and infant feeding. Lawson has told me that he came in contact with me in the wards after my return from France in 1919, but these encounters must have been only occasional, for at that time I had no clinical duties and my visits to the wards were only when I was delegated to teach. My knowledge and appreciation of Lawson would not have been long delayed, had I not been called away to the Yale Medical School in 1921, not to return until 1927 after Dr. Howland's death, so tragic to his pupils and friends.

My observation is that most interns on leaving the hospital for private practice become so absorbed by it that they participate less and less in the intellectual activities of their alma mater and tend, as the practical demands on their interest and time increase, to fall away altogether. Some have no interest beyond what might be called the operation of medical care; others with the spirit and qualities for scientific inquiry lack the surplus energy or time. Plenty of practitioners belong in academic medicine and we all can think of men in academic medicine who really belonged in practice from the day of their birth. There are, however, occasional superbeings who have been able to carry on a most extensive practice and to combine with it investigative work of great value. Lawson belongs to this group of unusual men.

Very soon after he had begun work as a practicing pediatrician his scientific studies began and it is noteworthy that the subjects which he chose were concerned with fundamental knowledge. His first paper, published with Benjamin Kramer in 1923, reported on the potassium content of human serum. In that same year and the following he was engaged with Emmett Holt, Orr, and Boone on investigations of the calcium and phosphorus metabolism of rickets which have a present-day importance. In 1924 he attempted to

protect infants against dysentery by the parenteral use of vaccine, and in 1927 a similar attempt was made with vaccine given orally. In the same year he undertook the ambitious project of curing rickets by an intramuscular injection of a cod liver oil derivative. This last piece of work has a sidelight because Lawson, needing a biochemist, obtained the cooperation of Charles Bills, who was at that time a postgraduate student under E. V. McCollum in the Johns Hopkins School of Hygiene. The interest which Lawson aroused in Bills concerning the nature of vitamin D was the immediate cause of the latter's entrance into steroid chemistry and not only determined the course of his career but was also the starting point for his notable discoveries. Another study which should be mentioned, performed a little later with Dr. Frank Ford, is the meticulous investigation of three patients who showed a curious insensitivity to pain. It is to be noted that in these early studies Lawson apprenticed himself to men of special experience from whom he could learn to penetrate beneath the surface.

In the period between 1921 and 1927, Lawson's bibliography shows the effect of his growing practice, or perhaps the lack of entree to the laboratory activities of the Harriet Lane, for his studies took the form of reviews. These reviews were responses to requests from Dr. Louis Hamman, but I think they represent attempts on Lawson's part to become completely conversant with subjects of vital importance to him as a practicing pediatrician and, by committing his material to writing, to clarify his own mind. They were most excellent clear expositions of needed subjects.

My recognition of Lawson's abilities came about in this way. Dr. Fritz Talbott of Boston sent Lawson a child with epilepsy in process of being treated with the ketogenic diet. I recall that Lawson came to the Harriet Lane to make inquiry in regard to the composition of the diet. In short, Lawson showed such interest in the treatment of epilepsy by means of the ketogenic diet that I asked him to join Dr. Edward Bridge, who was then in charge of the Epilepsy Clinic at the Harriet Lane. This Lawson did. It was as a result of his work with Dr. Bridge that Lawson in 1937 wrote his study, "Epilepsy in Childhood: I. A Statistical Study of Clinical Types; II. The Incidence of Remissions; III. Results With the Ketogenic Diet." This paper was a notable one and showed the care with which Lawson investigated the records and particularly the soundness of his conclusions. The detachment and common sense with which he approached his problem were particularly impressive. His work in the Epilepsy Clinic brought me into close contact with him and it was then that his potentiality in an academic atmosphere became apparent.

In 1935 Dr. Bridge left the Johns Hopkins Medical School to become Professor of Experimental Pediatrics at the University of Buffalo. I then offered Lawson the directorship of the Epilepsy Clinic, but he immediately declined. He said that he would prefer to work in some less depressing field. Of course the treatment of epilepsy was about to flower beyond imagination because of the development of new drugs, but that could not be envisaged at the time.

Unlike epilepsy it was plainly evident in 1935 that great advances were about to be made in endocrinology.* Scientific knowledge had developed just enough to stimulate speculation and not enough to control it. Falta had introduced the idea of multiple endocrine deficiencies, though his evidence was little more than guesswork. Under the leadership of men like Lewellys Barker of Johns Hopkins and Timme of New York endocrinologists were making diagnoses of complex endocrine disturbances from little more than conjecture and remarkable results from the administration of various hormone preparations by mouth were being reported. The practice of endocrinology was becoming very profitable. Lawson has told me that reading Engelbach's book on endocrinology late at night he became so disgusted

*The birth and development of scientific endocrinology have been traced by Lawson in his presidential address delivered before the Society of Endocrinology in 1957.

that he threw it across the room. The children suffering from endocrine disturbances in the Harriet Lane outpatient department were being treated by the various doctors to whom they happened to be assigned. There were no organization and no planned study. It was obviously necessary to place the study and treatment of these children on a sound basis and the only way in which this could be accomplished was to establish a special clinic devoted to the study of endocrinologic disorders. Anxious to place Lawson at the head of some important section of the Department, I offered him the directorship of the Endocrine Clinic. He replied by inquiring if I wished to make him a charlatan. In spite of misgivings that the road led to moral depravity Lawson accepted my offer.

From 1935 to 1946, Lawson continued to practice and at the same time to carry on his research and teaching in pediatric endocrinology. He was aided in his scientific work by a generous grant from the Commonwealth Fund, but this did not include salary and he received none from the Johns Hopkins University. At first he concentrated on the study of children suffering from thyroid deficiency, where he knew he was on solid ground, and he used to take me into his laboratory to show me his exquisite graphic studies of his patients. The graphs, he told me, he worked out between midnight and 2 A.M. I can recall my astonishment that he had the energy to do this after his daily marathon. Gradually his studies extended to other forms of endocrine disturbances as the patients exhibiting them came under his notice.

Dr. Francis Schwentker who succeeded me in 1946 was able to offer Lawson a "full-time" position in the Department. This Lawson accepted, although it meant a considerable reduction in income.

Shortly after he had made the decision to commit himself to academic medicine I remember that he stopped me in the hall of the Harriet Lane to express his relief. His new position was unique, "full time" at its best, for he had no fixed duties outside his own clinic. He now had complete liberty, liberty to read, to study, to enter the laboratory and to think. In short his only compulsion was to do those things which he most liked to do. No club behind, ahead nothing but carrots unlimited.

Lawson continued to head the Endocrine Clinic until the present time. In 1942 he was promoted from Assistant to Associate Professor and in 1957 to a full professorship. In 1953 he received the Borden Award of the American Academy of Pediatrics and in 1955 the Francis Amory Prize of the American Academy of Arts and Sciences.

I have often caught myself boasting that I discovered Lawson. Nothing could be further from the truth; he discovered himself. He was an indigenous plant springing up in my garden. All that I did was to furnish him his own plot of ground on which he proceeded to grow like Jonah's gourd. Dr. Schwentker fertilized the soil and pulled out all the competitive weeds.

I shall now make some scattered comments.

Lawson's studies have been based on the most meticulous investigations of his patients, not on the literature, as is the case with so many others. For this reason, his work has a particular authority.

The method which Lawson has employed is that which von Pirquet used with such brilliance in his studies on allergy and also measles, the graphic method. All data collected are represented visually on running charts which show their qualitative, quantitative, and temporal relationships. They are accompanied by timed photographs of the patients themselves. The whole produces a statistical record, the significance of which is apparent at a glance.

When I was a student in New York City, the late Dr. Holt used to repeat that only in private practice could one learn prognosis. Undoubtedly a general experience in prognosis is thrust on the practitioner in a way denied to physicians whose work is confined to hospitals and dispensaries where individual patients are lost from both sight and mind in the overcrowded shifting populations. But the special clinics assigned to

the study of particular diseases are the ultimate sources of knowledge concerning the outcome of disease. An endocrine clinic lends itself particularly to long-term study, for the patients are rare enough and, also, peculiar enough, to stand out clearly in the mind, and they tend to keep returning spontaneously because they continually require help. Moreover, in endocrinology, studies extending through the period of childhood into adult life and including the period of reproduction have a particular importance and, indeed, the question of longevity is involved. Lawson's clinic has been distinguished by its long-term studies. In the systematic "follow-up" of his patients, the new data are entered on graphic charts which now in many instances cover years. These graphic charts are already a visual library of endocrinology. If the clinic is perpetuated indefinitely, as I hope will be the case, these records will have the same importance in contributing to the knowledge of disease that biographies have in the formation of history.

Lawson has said to me more than once that he would not have been deprived of his 25 years in practice for anything. It is interesting to speculate what the influence of this has been on his final development, and what would have happened had he committed himself to "full time" at the beginning. All who have worked with Lawson agree that he possesses every quality for leadership in academic medicine. Had he chosen to put his life there, when Dr. Howland offered the assistant residency, he would have soon more than qualified to become the head of any one of the foremost pediatric clinics in the country. I think, however, that he never had any ambition for this. From the way in which Dr. Howland's clinic was organized in 1922, Lawson would have undoubtedly been drawn into the vortex of biochemical research and become a biochemist in his own right. The direct approach to his problem via the test tube would then have become the primary motif. However, Lawson's associates have expressed to me their surprise over his knowledge of steroid chemistry, picked up without direct laboratory experience.

It is difficult to distinguish in just what ways Lawson's experience in practice has helped him in his final development, but it obviously has had a great influence on its course. It has caused him to approach the problems of his patients primarily as a physician, secondarily as a scientist. It has enabled him to view his human subject in totality and to move to the particular from the wide perspective of the general. The welfare of the patient has always been the primary consideration. Lawson's assistants have told me that he was never willing to gamble for the sake of new knowledge. This has meant that he never embarked on a treatment which had not been shown to be sound or whose use was not fully justified on logical grounds after careful examination into all its aspects.

The consciousness of years of selfless work must be a great satisfaction to a practicing physician, but its value is so diffused into the mass of humanity that the only one capable of estimating it objectively is the recording angel. But Lawson's work as a student and investigator is open to view. I do not know that he could have obtained more eminence, had he earlier started differently, for he is known the world over as an authority and a pioneer in child endocrinology. His abilities to investigate and to teach are so unusual, however, that I for one regret that medical science could not have had him all the time.

Lawson's clinic has grown as he has grown until it has attracted students from far-distant countries. A great teacher influences not only by his ideas, but also by his standards and ideals, and fires his pupils with his spirit quite as much as he feeds their minds. I am sure that Lawson's attitude has had a profound effect on his pupils and that they have gone from him not only wiser, but better, human beings. These students trained under him will extend knowledge beyond that of their teacher. This is as it should be.

I shall leave the picture of Lawson at work in his clinic to one of his pupils, Dr. Bongiovanni; also to him Lawson's scholarship and the account of his contributions to

knowledge, including his book, after he took up endocrinology, for these latter reveal Lawson himself in a very special way and are far more important than the earlier studies which I have mentioned. I also leave to Dr. Bongiovanni the picture of Lawson as a glorious human being, outgoing, friendly and jolly, always his natural self unspoiled by success.

EDWARDS A. PARK, M.D.

# *Lawson Wilkins*

IN THE dedication of his widely acclaimed book *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence,* Lawson Wilkins pays tribute to the roles of the father (his own), the family physician (again his father), the pediatrician (E. A. Park), and the endocrinologist (Fuller Albright). Add to these roles that of teacher and Lawson Wilkins has himself brought fulfillment to all as few men of medicine are privileged to do. His indelible imprint is left on the many young people throughout the world who have been fortunate enough to come under his guidance. They are listed here and join in honoring this great American physician in his sixty-fifth year.

Dr. Park has outlined some of the early life and career of Lawson Wilkins. Others of his colleagues express their tributes on this occasion.

Wilburt C. Davison (Durham, North Carolina) expresses his perpetual "highest regard for his intellectual ability and his personality" and tells a little about his early career as a physician in the armed forces during World War I: "I have followed his career with great interest from 1917 when we went to France with the American Expeditionary Force, Lawson going as a buck private and I as a first lieutenant because I was a year senior to him. At Savenay, Lawson incurred the wrath of the top sergeant which is a dangerous thing to do in any man's army and Lawson was assigned to scything the grass in the courtyard of the Savenay Hospital but with instructions not to touch any of the daisies. Lawson has been frustrated ever since. Later in 1917 we were in the same area, he being with Base Hospital 18, the Hopkins Unit at Bazoilles, and I was with the First Army Laboratory in Neufchateau, seven miles away. Each Saturday, our outfit was marched to Bazoilles where Lawson and his fellow buck privates had to heat up enough water to give all of us a bath. One of my colleagues of the First Army Laboratory said he enjoyed a bath every Saturday, but he did not relish the idea of being ordered to take one. I am sure also that Lawson and the fellow buck privates in Base Hospital 18 did not enjoy boiling the water for hot showers for our outfit."

Horace Hodes (New York City) was associated with Lawson Wilkins in Baltimore "off and on for seventeen years" and recalls his dedication to teaching while engaged in active pediatric practice: "I recall with the greatest of pleasure the rounds which Lawson made while I was assistant resident at the Harriet Lane Home in 1932 and 1933. You may know that the house staff in the Harriet Lane Home always had a free hand and was under no compulsion to ask advice from older heads. Indeed, I am afraid there were times when the attending staff was made to feel rather superfluous. I need not tell you, however, that this was never the case with Lawson. We looked forward each day to his rounds and each day brought help and knowledge from him. We were always surprised at the breadth of his wisdom and his great good sense, all flavored with a kindly humor. It was no surprise to any of us that when he turned to research Lawson became the outstanding pediatric endocrinologist of his time. He did everything well and many have forgotten that his contributions to knowledge about epilepsy were of first rate importance."

Mitchell Rubin (Buffalo, New York) indicates the contributions of Lawson Wilkins to pediatrics: "His monumental

work and publications in pediatric endocrinology and his fine textbook on this subject stand as an oracle to all of us interested in the care of children. The frayed second edition of his text on our library shelf attests to its frequent use by staff members, residents, and medical students. Dr. Wilkins has brought order into an area where variability itself was a cause of chaos. Many children today actually owe their lives to Dr. Wilkins, who has given us such a broad understanding of the adrenogenital syndrome. I think it is fitting to call to the attention of pediatricians in general the fact that Dr. Wilkins began his studies and made many of his impressive contributions to the understanding of endocrine disorders of childhood when he was fully engaged in the active private practice of pediatrics. His capacity to carry on major scientific study while caring for large numbers of children in private practice should be an inspirtation and a stimulus both to the pediatric practitioner and to the investigator. Dr. Wilkins' constant willingness to be helpful to others needing his advice and judgment is another quality which endears him to great numbers of pediatricians throughout the country, a quality which has also defined him as an outstanding teacher in pediatrics."

Much as Lawson Wilkins protests that he is not a scientist, some of his contributions are basic and contradict his denial. Furthermore, he has provided great impetus to the pure scientist. Reginald Archibald of the Rockefeller Institute is moved to write: "Not only has Dr. Wilkins done more than any other man I know during the past two decades to dispel chaos and to systematize knowledge within the field of pediatric endocrinology, he has also been perhaps the most effective teacher in this field. Through his textbook . . . his Saturday morning clinics and his lectures both in Baltimore and throughout the country, he has made endocrinology a rational clinical science for many members of at least two generations of physicians who attended medical school at a time when there was almost no effective teaching or training in this field. Dr. Wilkins has always been forthright in his dealings with colleagues and patients; rational, conservative and effective with respect to his recommendations for therapy and productive as an investigator. The peace of mind which he has helped many patients acquire, the productivity and eminence of those who were once his junior colleagues and the untold gratitude of hundreds of physicians who have heard his lectures or read his book bear witness to the greatness of this man as a physician, as a teacher and writer, and as an investigator."

Alton Goldbloom, noting that "nothing is needed to enhance his already unique stature in the science of his field" praises the man: "What should be emphasized, however, is Lawson's extraordinary quality of humility which in the face of his great abilities endears him to all who know him. He has the gift of self effacement coupled with a disarming and charming shyness which give his utterances and his writings a deserved quality of authority. One of the greats in Pediatrics and I am proud to know him and to be his friend."

It has not been many years since Lawson Wilkins was propelled into world-wide eminence. Yet, in that time, those qualities which radiated the atmosphere of those working closely with him, in training or as colleagues, became quickly apparent to his audience abroad. Douglas Hubble, Professor of Child Health and Chairman of the Department of Pediatrics at the University of Birmingham, writes as follows: "Lawson Wilkins erupted into the European paediatric scene at Zurich in 1950. I don't know how many times he may have been in Europe since 1918 when, as a medical student from Johns Hopkins, he survived World War I in the United States P.B.I.* but for most of us here this was his brilliant first appearance. Friendly, receptive, patient, he sat by his great exhibit—which was indeed a preview of his book—ready to teach what he had himself learnt in a decade of un-

*Not protein-bound iodine.

rivalled experience. After Zurich his English friends sought out the company of Lawson and his gracious wife wherever they were to be found, and by some alchemy their personalities, no matter how inhibited, were kindled by the warmth of the Lawson Wilkins'. All the mental and physical qualities which one was later to know so well were there in 1950 in fine array. It is characteristic of him that he has always preferred fact to speculation, measurement to guesswork, and etiological to eponymous nomenclature. Painstaking accuracy has informed all his work and has been imposed on the succession of distinguished Associates and Fellows who have worked with him. To everything he has done, he has brought the same enormous gusto. Robust in physique, he can

'Tire the sun with talking
And chase him down the sky'

and yet work hard the next day.

"Confident of what he himself knows and has discovered, he is generous in his appreciation of other men's work. His reading ranges widely and he comes to the exploration of biological literature with a 'mind prepared' to recognise what is contributory to his own thinking. There are many authors on this side of the Atlantic whose papers would not have entered the American bibliographies if Lawson had not read them. How many of us, whether paediatricians or endocrinologists, would have heard of Jost, if Lawson had not borrowed his French horn and blown a stentorian blast upon it?

"He found the subject of paediatric endocrinology a murky backwater. He widened its banks, dredged its bed, cut down the weeds, and linked its stream with the main current of endocrinology whose waters have been freshened and invigorated by their youthful tributary. This is his achievement. Paediatric endocrinology is still a small subject but it is now an established specialty and Lawson's work has both the admiration of paediatricians and the respect of endocrinologists. He has become a star of American Medicine, worthy to be named with Fuller Albright, Gamble, and Loeb and not unworthy of his father-figures at Johns Hopkins, William Osler and Edwards Park."

The students of Lawson Wilkins join in wishing him a long life of further usefulness and the opportunity to enjoy the fruits of his work:

| | |
|---|---|
| David S. Alexander | Miami |
| Henning Anderson | Denmark |
| Cesar Bergada | Miami |
| Jean Bertrand | France |
| Robert M. Blizzard | Columbus |
| Alfred M. Bongiovanni | Philadelphia |
| Jose Cara | Syracuse, N. Y. |
| George W. Clayton | Houston |
| William W. Cleveland | Miami |
| Raphael R. David | Miami |
| Enrique Dulanto | Mexico |
| Walter R. Eberlein | Philadelphia |
| John P. Eckert | Miami |
| Walter Fleischmann | Memphis |
| Lytt I. Gardner | Syracuse, N. Y. |
| Jean-Luc de Gennes | France |
| John Gerrard | Saskatchewan |
| Orville C. Green | Columbus |
| Melvin M. Grumbach | New York |
| Heskel Haddad | Bethesda |
| Fouad Hameoui | Lebanon |
| Joan G. Hampson | Baltimore |
| John L. Hampson | Baltimore |
| Jean Heimendinger | Switzerland |
| Gerald H. Holman | Saskatchewan |
| Edouard Juillard | Switzerland |
| Gordon Kennedy | England |
| Robert Klein | Pittsburgh |
| Roger A. Lewis | India |
| Salvadore de Majo | Argentina |
| M. Mencer Martin | Washington, D. C. |
| Claude J. Migeon | Baltimore |
| John Money | Baltimore |
| H. David Mosier | Los Angeles |
| Marija Nikezic | Yugoslavia |
| Constantine Papadatos | Greece |
| Eugenia Rosemberg | Shrewsbury, Mass. |
| John W. Shaffer | Baltimore |
| Thomas Shepard | Seattle |
| Samuel Silverman | Perth Amboy, N. J. |
| David W. Smith | Madison |
| Robert S. Stempfel | Durham |
| Gloria Stewart | New Orleans |
| Judson J. Van Wyk | Chapel Hill |

ALFRED M. BONGIOVANNI, M.D.

# *The relationship of acidosis and growth retardation*

**Robert E. Cooke, M.D.,* Douglas G. Boyden, M.D.,****

**and Evelyn Haller, B.S.**

BALTIMORE, MD.

THE rate of growth is dependent upon the interplay of a number of poorly defined factors.[1] Growth retardation or arrest is observed in such diverse conditions as undernutrition, congenital heart disease of the cyanotic or acyanotic types, chronic pulmonary disease, steroid therapy, renal disease, and many others.[2-7] Although a deficiency of protoplasmic precursors or regulating hormones may be responsible in a few instances, complex disturbances of cellular function are probably responsible in the majority of instances.

The cause of the retardation of growth observed in certain cases of chronic renal disease is undetermined. A strong association between growth failure and acidosis in renal disease has been shown by a number of workers.[8-11]

The experiments reported here represent a portion of the studies undertaken several years ago to evaluate the role of acidosis in the production of growth retardation. Chronic acidosis (depression of pH) was produced in rats by lowering of the serum bicarbonate concentration by loading with ammonium chloride as well as by administration of renal carbonic anhydrase inhibitor (acetazolamide) or by raising the serum carbonic acid concentration. Weight gain and linear growth were recorded on a carefully measured food intake. These experiments simulate some of the aspects of renal acidosis in children.

*From the Departments of Pediatrics, The Johns Hopkins University School of Medicine and the Yale University School of Medicine.*

**Address, Department of Pediatrics, The Johns Hopkins Hospital, Baltimore 5, Md.*

***This study represents a portion of the thesis submitted for the degree of Doctor of Medicine, Yale University School of Medicine, 1955. Aided by a grant from the Playtex Park Research Institute.*

## METHODS AND PROCEDURE

Thirty-day-old male albino rats of the Sprague Dawley strain were used and were maintained in individual cages 7 by 7 by 7 inches of ½ inch wire mesh. Water and Purina fox chow were allowed ad libitum for 8 to 10 days to determine the normal rate of growth and to insure that all the animals were healthy. Environmental temperature was maintained at 25° C. with only slight variation.

After this preliminary period the rats were arbitrarily separated into four groups and each animal was paired with the one nearest to it in weight within a given group. At this time the animals' ears were notched to give a positive means of identification.

The feed in all cases was ground fox chow to which the necessary amount of the materials under study was added. This feed was

given to the rats in small round tins 3 by 1½ inches in size with closed covers which were wired to the floor of the cage. Access to the feed was provided by a hole 1½ inches in diameter surrounded by a large flange which admitted only the rat's head.

All animals received water ad libitum. Experimental animals received feed ad libitum. This feed was weighed daily and a like amount of feed was given to the control animals. Thus, the control animals received in one day the amount of feed consumed during the previous day by the experimental animals. Allowances were made for wastage. No allowance was made for 4 per cent of the diet which in the experimental animals was electrolyte (ammonium chloride) in groups I B and II. It was considered that this amount of feed was not significant, especially since feed was weighed only to 1 Gm. Possible difficulties arising from this procedure are discussed later. All animals were weighed every other day on a torsion balance accurate to 0.1 gram. At intervals during the early experimental period samples of tail blood were collected under oil for chloride analysis to determine the level of acidosis. Concentration of the acid-producing substances in the diet was modified upward until the end of the experimental period. All animals showing signs of upper respiratory infection were excluded from the experiment.

**Experimental groups.**

*I A. Seven experimental animals and 7 controls, unilateral nephrectomy.* At the start of this experiment the animals were anesthetized with ether. The left kidney was removed through a left lumbar paravertebral incision. There was little hemorrhage and the animals withstood the procedure well. Four experimental and 2 control animals were sacrificed at the end of the experiment.

*I B. Six experimental animals and 6 controls, unilateral nephrectomy and 4 per cent ammonium chloride.* The animals in this group underwent the same operative procedure as those in Group I A. These rats were then placed on a diet containing 4 per cent ammonium chloride. At the end of the experiment 4 experimental animals and 2 controls were sacrificed for serum, muscle, and bone analyses, and survivors were placed on a normal diet.

*II. Eleven experimental animals and 11 controls, 4 per cent ammonium chloride and 0.1 per cent acetazolamide.* These animals were started on a diet containing 1 per cent ammonium chloride. This diet did not produce a satisfactory acidosis as shown by serum chloride determinations. The amount of the ammonium chloride was increased to 2 per cent and 0.05 per cent acetazolamide was added. This diet also produced an unsatisfactory acidosis, and consequently the amount of both these substances was doubled. The final diet contained 4 per cent ammonium chloride and 0.1 per cent acetazolamide. At the termination of the experiment 7 experimental animals and 3 controls were sacrificed for analysis. Survivors were placed on a neutral diet which contained 4 per cent ammonium chloride, 0.1 per cent acetazolamide, 2 per cent potassium bicarbonate, and 7 per cent sodium bicarbonate.

*III. Twelve experimental animals and 12 controls, 13 per cent carbon dioxide.* The experimental animals in this group were maintained in a clear lucite box, approximately 2 by 2 by 2 feet. One side was removable for access to the individual cages in which the rats were kept. This side was sealed by a rubber gasket. The seal was broken once each day for weighing feed and animals and for cleaning the cage. Excess moisture in the chamber was absorbed by a beaker of concentrated sulfuric acid. Compressed carbon dioxide and compressed room air were led into a small mixing chamber by rubber tubing and thence into the center of the chamber. This atmosphere was analyzed once each day for carbon dioxide. The average concentration of carbon dioxide in the air was 13 per cent. The average concentration of oxygen was 17 per cent. At the termination of this experiment, 7 experimental animals and 3 controls were selected for analysis. These animals were anesthetized and their heads were placed in a cone of stiff paper, to which the gas mixture was led by rubber

tubing. This device served to maintain the serum carbon dioxide tension while the animals were being exsanguinated. The survivors were then maintained in room air.

*IV. Ten experimental animals and 10 controls, 0.4 per cent acetazolamide.* The experimental animals in this group were placed on a diet of 0.2 per cent acetazolamide. Two pairs of animals were sacrificed for serum determinations of sodium, potassium, carbon dioxide, and chloride. These indicated only a slight degree of acidosis, so the amount of acetazolamide in the diet was doubled and maintained at 0.4 per cent. After 42 days, all animals were sacrificed.

## RESULTS

**General observations.** One animal in Group I and 3 in Group II showed signs of upper respiratory infection manifested by wheezing, sniffling, and lethargy. These animals were destroyed to prevent the spread of the illness, and they were excluded from the results. The other animals were well maintained under various acid-producing regimens. The experimental animals in Group III suffered anorexia while in 13 per cent carbon dioxide and did not eat normal amounts of food. This decreased food intake caused the control animals in this group to be ravenously hungry and to gain less weight than the controls of other groups.

There was no observable difference in activity between experimental and control animals of Groups I, II, and IV. The experimental animals in these groups were not obviously hyperpneic. The animals in Group III while in 13 per cent carbon dioxide usually lay curled up in their cages and were obviously hyperventilating. As soon as the cages were removed from carbon dioxide for

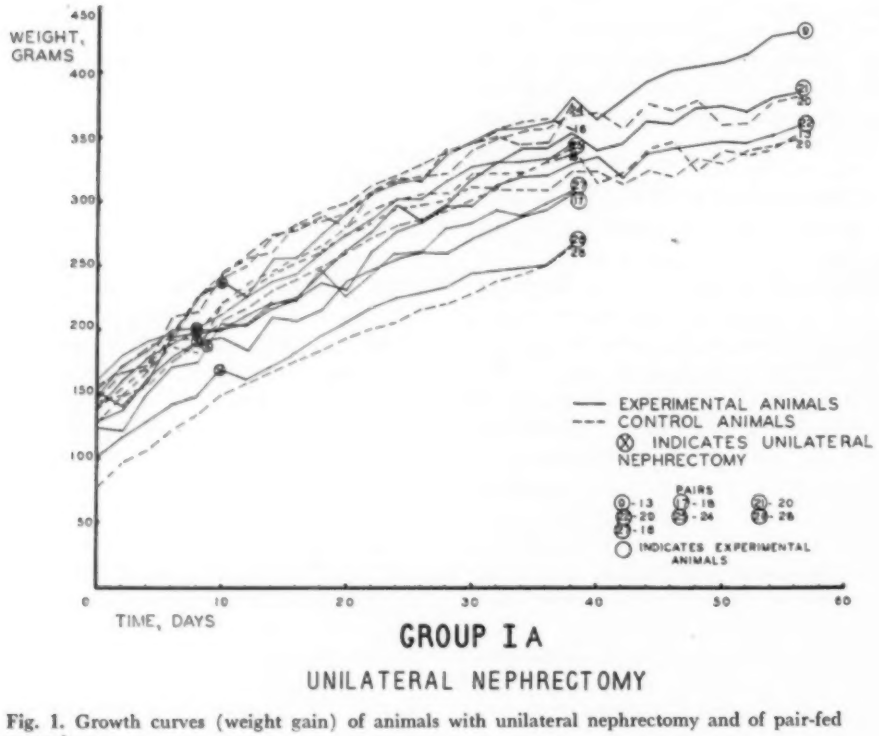



**Fig. 1.** Growth curves (weight gain) of animals with unilateral nephrectomy and of pair-fed controls.

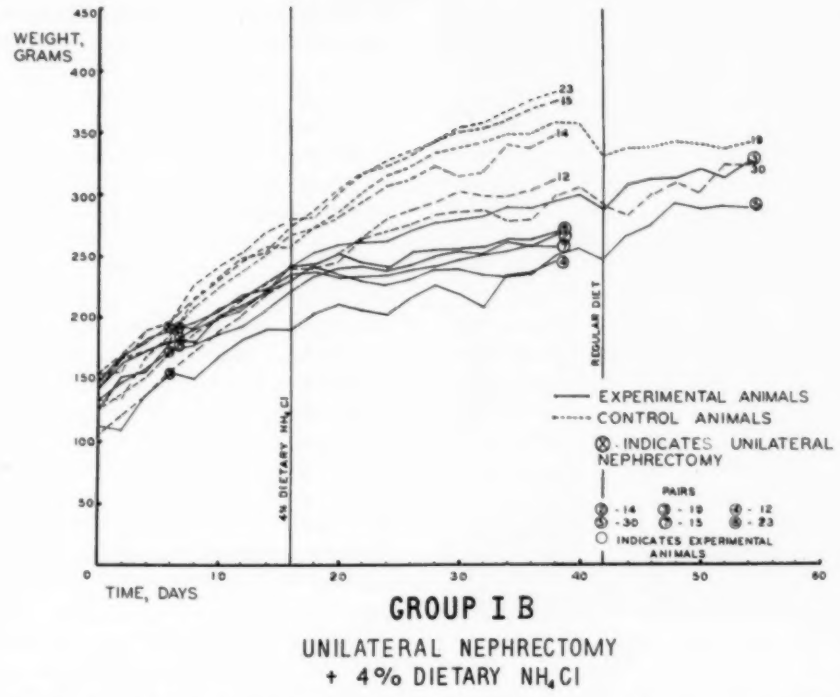



**Fig. 2.** Growth curves (weight gain) of animals with unilateral nephrectomy, ingesting a diet containing 0.4 per cent ammonium chloride, and of pair-fed controls.

cleaning, the animals cleaned themselves off thoroughly, scampered about in their cages, apparently enjoying the fresh air. The controls showed activity comparable to that of the animals in Groups I and II.

The important data from all groups are summarized in Tables I, II, and III. Table I shows the chemical changes in the serum. Table II presents the food intake, weight gain, and nutritional efficiency (grams gained per gram of feed). Table III indicates the weights of the kidneys, adrenals, and thyroid. In Groups I A and I B the weight of only the remaining kidney is given. Muscle and bone analyses were also carried out in each group. Muscle analyses of the experimental animals did not differ markedly from those of the controls and do not help in understanding the growth defect. Unfortunately, the variation in bone analyses is large, partly because of technical difficulties. Although statistically significant differences existed in some groups, the data are not presented and no conclusions are based on the findings of bone analyses.

**Group I A.** Unilateral nephrectomy produced little change in electrolyte concentrations in the serum, and pH was not depressed. The rate of weight gain (Fig. 1) and linear growth (as determined by the length of the femur) of the experimental animals did not differ significantly from that of the controls. The growth curves demonstrate that recovery of rate of gain of weight occurred approximately 12 days after nephrectomy. At the end of the study (32 days) both groups of animals weighed approximately the same and were growing at the same rate. Organ weights indicated that the remaining kidney had enlarged. Thus, the experimental animals had 71 per cent of the renal mass of their normal controls per unit of body weight. The adrenal weights were comparable in the two groups. Thyroid weights were greater in

the experimental animals, but the number of specimens was small.

**Group I B.** The addition of 4 per cent ammonium chloride to the diet of rats with unilateral nephrectomy produced a slight depression of carbon dioxide content of the serum and a slight elevation of chloride level. The acidosis was well compensated since pH after 32 days was not lowered significantly below that of the pair-fed controls. However, weight gain was markedly depressed (Fig. 2). The control animals gained 1.7 times as much weight as the experimental animals on the same food intake. The two groups of animals gained at the same rate until the experimental animals underwent nephrectomy. At that time these animals suffered a slight lag in growth but soon recovered, and 8 days postoperatively the addition of 4 per cent ammonium chloride led to immediate slowing of weight gain. Sixty per cent of their total increment in weight was attained in the 10 days from nephrectomy to the addition of ammonium chloride to the diet and only 40 per cent in the 22 days thereafter. The rate of linear growth was also significantly depressed as determined by femur length.

The remaining kidney had hypertrophied to 64 per cent of the weight of the two kidneys in the controls. When the weight of the kidney was related to total body weight, the experimental animals had 82 per cent of the renal mass of the normal animals. The relative weight of the adrenal was larger in the experimental animals. There was a small difference in thyroid weights.

Histologic examination of the thyroid and adrenals was unremarkable in Groups **I A** and **I B.** Sections of kidney in both groups of experimental animals showed evidence of hypertrophy characterized by increase in size of the glomeruli and dilatation of the tubules. Changes were possibly greater in Group **I B** than **I A.**

**Group II.** The addition of 2 per cent am-

**Fig. 3.** Growth curves (weight gain) of animals ingesting a diet containing 4 per cent ammonium chloride and 0.1 per cent acetazolamide and of pair-fed controls.

monium chloride and 0.05 per cent acetazolamide failed to produce acidosis or significant depression of weight gain (Fig. 3). The addition of 4 per cent ammonium chloride and 0.1 per cent acetazolamide led to a significant and sustained and not completely compensated hyperchloremic acidosis. Serum pH averaged 7.22 as contrasted with 7.46 in the controls. Levels of sodium, potassium, and inorganic phosphorus in the serum were not altered.

Accompanying the acidosis was a marked depression of weight gain. The nutritional efficiency of the experimental animals averaged 0.154 as contrasted with 0.231 in the controls. Femur lengths were markedly reduced in the experimental group.

Although in absolute terms the organ weights did not differ markedly per unit of body weight, the kidneys and adrenals were significantly larger in the acidotic animals. However, little change from the normal histologic picture was observed in the kidney, adrenal, and thyroid. Sections of the distal end of the femur showed some changes in every experimental animal. These changes were only moderate in degree and consisted of osteoporosis in 3 animals and osteitis fibrosa in 4 (Fig. 4). Radiologic examination of one pair of animals disclosed no marked skeletal abnormalities.

**Group III.** Exposure of rats to an atmosphere containing 13 per cent carbon dioxide for 38 days produced a profound and only partly compensated respiratory acidosis. The carbon dioxide content was elevated to 50 mM. per liter, and the chloride was depressed to 78 mEq. per liter. Serum pH averaged 7.11.

Accompanying the acidosis was the most severe depression of weight gain observed (Fig. 5). The feed intake was decreased from the usual 20 or so grams per day to 12 or 13. Control animals, though limited to the same feed consumption, gained 120.8 grams contrasted with 39.1 in the experimental group. The nutritional efficiency of the controls was three times that of the experimentals (0.252 versus 0.080). During a 16 day recovery period the nutritional efficiency of the experimental animals rose to 0.279 and the slope of the growth curve became normal. The femur length was markedly decreased in the acidotic group as compared with the controls (27.0 mm. versus 29.4 mm.).

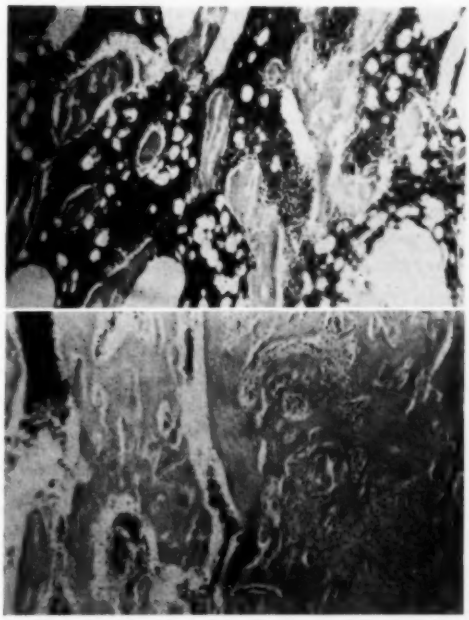


**Fig. 4.** Histologic appearance of the distal end of the femur in control (above) and acidotic animal of Group II (below). Osteitis fibrosa can be seen in the acidotic animal.

Although in absolute terms the organ weights did not differ markedly per unit of body weight, the kidneys and adrenals were significantly larger in the acidotic animals. No change from the normal was observed histologically in the kidney, adrenal, or thyroid glands. Sections of the distal end of the femur revealed osteoporosis and mild osteitis fibrosa in a few animals. Radiologic examination of one pair of animals showed a decreased radiodensity of the appendicular skeleton in the experimental animal. No rachitic changes were noted (Fig. 6).

**Group IV.** The addition of 0.4 per cent acetazolamide to the diet produced a sustained and incompletely compensated hyperchloremic acidosis. Serum pH averaged 7.13

as contrasted with 7.36 in the controls. Although sodium and potassium concentrations were normal, serum inorganic phosphorus was significantly elevated to 11.1 mg. per cent.

Despite the presence of an acidosis almost as severe as in Groups II and III, there was no depression of weight gain (Fig. 7) or linear growth. The nutritional efficiency was 0.279 in the acidotic animals as compared with 0.281 in the controls. The femur lengths differed by only 0.1 mm. Likewise, there were no significant differences in organ weights or histologic appearance.

## DISCUSSION

These experiments confirm previous findings that growth retardation results from marked alterations in dietary electrolyte intake. Meneely[12] observed that diets containing 9.8 per cent sodium chloride caused decreased weight gain over a 20 week period in rats. He advanced no explanation for this phenomenon. Addis and associates[13] observed that 2 per cent calcium chloride in the diet produced retarded growth as measured by both weight gain and skeletal length in rats. Jaffe and co-workers[14] showed that ammonium chloride ingestion with a low calcium diet retarded growth in dogs. Burns[15] showed that 0.1 per cent ammonium chloride in the diet of rats caused decreased growth.

None of the above workers utilized a paired feeding technique in their experiments so that nutritional factors undoubtedly complicated the findings.

Correlation between depression of pH and retardation of growth was not invariable in the present experiments. Severe retardation of growth and weight gain occurred in the absence of significant acidosis in the animals with unilateral nephrectomy that received 4 per cent ammonium chloride in the diet (Group I B). Loading with ammonium chloride may be compensated by synthesis of ammonia and excretion of acid urine. If the load is excessive, fixed cations such as sodium, potassium, calcium, or magnesium

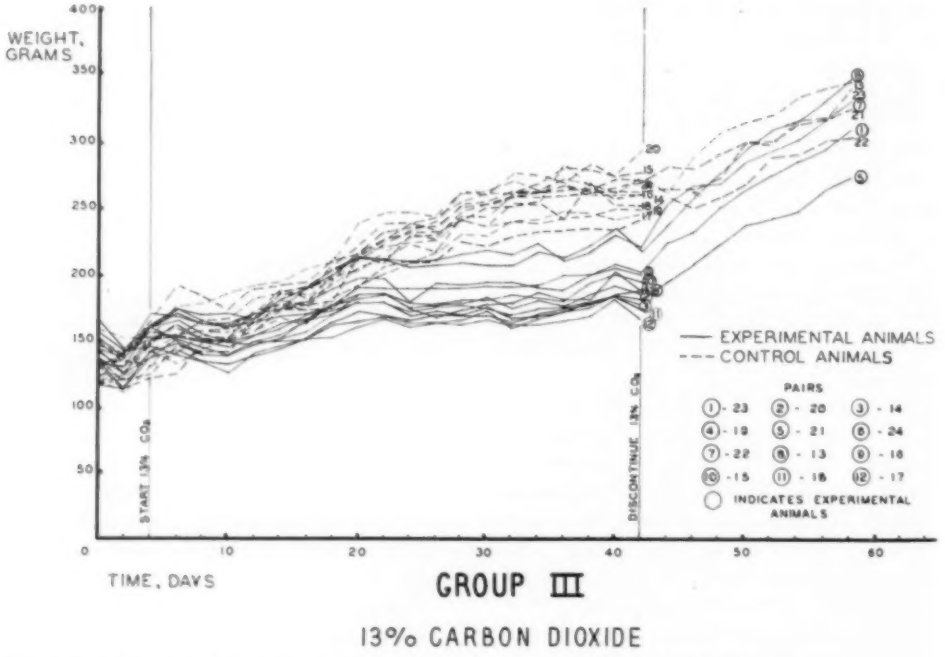



**Fig. 5.** Growth curves (weight gain) of animals existing in atmosphere containing 13 per cent carbon dioxide and of pair-fed controls.

**Fig. 6.** Roentgenogram of animal existing in atmosphere containing 13 per cent carbon dioxide (right) and pair-fed control (left). The differences in size and density of the appendicular skeleton are obvious.

may be lost. Despite normal serum pH which can be sustained by respiratory compensation, large amounts of fixed cations may be lost from reservoirs such as bone.[16, 17] Such losses may possibly lead to growth arrest by diminishing the supply of cellular and matrix components.

Losses of cations might possibly have led to increase in adrenocortical secretion. The large size of the adrenals in the animals receiving ammonium chloride is suggestive evidence for such a mechanism. However, proper evaluation of the significance of changes in organ sizes is difficult in the presence of growth disturbance.

Loss of nitrogen through renal ammonia synthesis cannot be responsible for the retardation of growth observed since extra nitrogen was present in the diet as ammonium ion which can be utilized by the rat to sustain nitrogen balance. A systematic error in paired feeding technique can hardly be responsible for the results reported. A similar growth pattern of experimental and control animals in Groups I A and I V eliminates the possibility that the abnormal feeding habits of the pair-fed controls are respon-

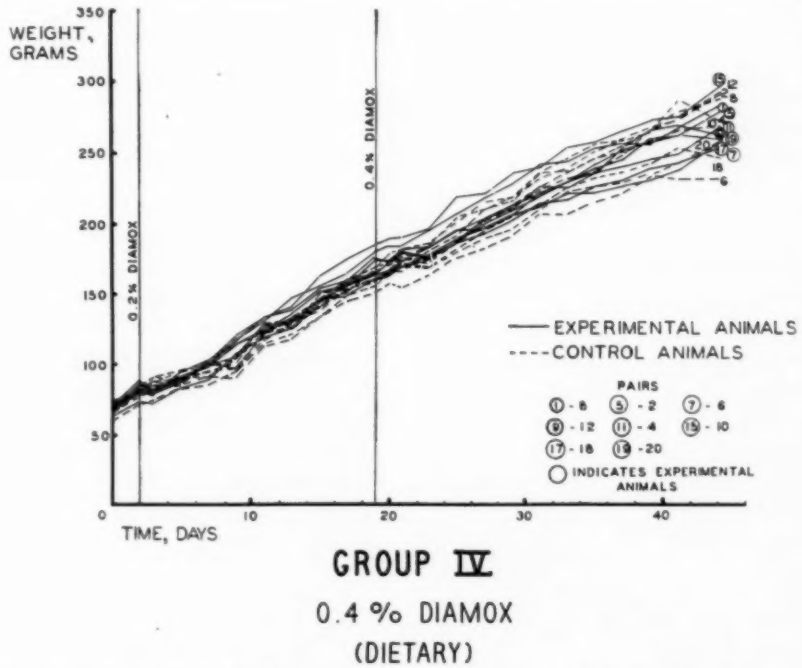



**Fig. 7.** Growth curves (weight gain) of animals ingesting a diet containing 0.4 per cent acetazolamide and pair-fed controls.

**Table I.** Summary of chemical data

| *Group* | *Number of animals* | *Serum pH* | *Serum electrolytes* | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Na (mEq. per liter)* | *K (mEq. per liter)* | *$CO_2$ (mEq. per liter)* | *Cl (mEq. per liter)* | *P (mg. %)* |
| IA | 4* | 7.33 ± .42† | 143.1 ± .4 | 4.3 ± .3 | 27.3 ± 2.1 | 96.9 ± .8 | 8.8 ± .3 |
| | 2 | 7.33 ± .01 | 143.4 ± .7 | 4.1 ± .0 | 29.4 ± 1.9 | 95.9 ± 1.2 | 9.3 ± .4 |
| IB | 4 | 7.42 ± .06 | 141.9 ± .7 | 4.0 ± .2 | 22.7 ± 2.2 | 103.1 ± 3.2 | 8.3 ± .2 |
| | 2 | 7.46 ± .01 | 141.1 ± 1.6 | 4.7 ± .1 | 24.9 ± 1.4 | 96.5 ± 1.1 | 8.3 ± .5 |
| II | 7 | 7.22 ± .03 | 143.9 ± .4 | 4.2 ± .1 | 17.2 ± .8 | 107.3 ± 1.4 | 9.5 ± .1 |
| | 3 | 7.46 ± .02 | 142.3 ± .5 | 4.3 ± .1 | 23.9 ± .2 | 98.0 ± .9 | 8.5 ± .2 |
| III | 7 | 7.11 ± .03 | 145.4 ± .5 | 3.6 ± .3 | 50.0 ± 1.5 | 78.1 ± .7 | 7.1 ± .4 |
| | 3 | 7.35 ± .03 | 143.2 ± 1.6 | 3.8 ± .03 | 23.6 ± .7 | 99.9 ± .6 | 8.6 ± .4 |
| IV | 8 | 7.13 ± .01 | 144.0 ± .5 | 4.0 ± .2 | 19.8 ± .5 | 108.4 ± .5 | 11.1 ± .1 |
| | 8 | 7.36 ± .00 | 142.0 ± .6 | 4.4 ± .2 | 24.7 ± .6 | 99.8 ± .7 | 9.1 ± .2 |

*Experimental values underlined.

†Standard error.

**Table II.** Summary of growth data

| *Group* | *Number of animals* | *Duration (days)* | *Weight gain (grams)* | *Grams gain / Grams feed* | *Length of femur (inches)* |
|---|---|---|---|---|---|
| IA | 7* | 32 | 140.6 ± 8.5† | .205 ± .010 | 30.0 ± .4 |
| | 7 | | 150.1 ± 7.8 | .222 ± .013 | 30.5 ± .1 |
| IB | 6 | 32 | 96.2 ± 6.9 | .141 ± .013 | 29.1 ± .3 |
| | 6 | | 166.4 ± 8.4 | .202 ± .011 | 30.8 ± .4 |
| II | 11 | 40 | 125.8 ± 6.8 | .154 ± .009 | 29.1 ± .3 |
| | 11 | | 191.1 ± 7.3 | .231 ± .009 | 31.6 ± .1 |
| III | 12 | 38 | 39.1 ± 3.7 | .080 ± .007 | 27.0 ± .2 |
| | 12 | | 120.8 ± 4.3 | .252 ± .007 | 29.4 ± .3 |
| IV | 8 | 42 | 184.8 ± 4.5 | .279 ± .007 | 28.9 ± .04 |
| | 8 | | 185.9 ± 7.4 | .281 ± .009 | 28.8 ± .04 |

*Experimental values underlined.

†Standard error.

sible for observed differences. Likewise, it is unlikely that the 4 per cent decrease in nutriment that occurred in Groups I B and I I could be responsible. A 4 per cent deficit over 40 days amounts to approximately 30 grams of feed. If a nutritional efficiency of 0.3 is assumed (higher than observed in any control group) a weight difference of only 9.0 grams could be accounted for in this way as contrasted with an observed difference of 50 to 70 grams.

The mechanism by which respiratory acidosis leads to growth retardation is even more obscure. The initial renal response to elevation of the carbon dioxide tension of body fluids is the excretion of anion, especially chloride in excess of sodium. Potassium excretion may be elevated for a brief period, but as ammonium excretion rises potassium retention may occur. A low pH by increasing ionized calcium slightly may lead to a considerable increase in filtered calcium and possibly in calcium excretion which persists throughout the period of acidosis. No evidence of rachitic change was observed however. Losses of nitrogen in the urine may

**Table III.** Summary of organ data

| Group | Number of animals | Weight of kidney (Gm.) | | Weight of adrenal (mg.) | | Weight of thyroid (mg.) | |
|---|---|---|---|---|---|---|---|
| | | Absolute | Per 100-Gm. rat | Absolute | Per 100-Gm. rat | Absolute | Per 100-Gm. rat |
| IA | 4* | 1.7090 ± .0680† | .560 ± .038 | 62.8 ± 1.8 | 20.5 ± .8 | 28.0 ± 1.0 | 9.2 ± .6 |
| | 2 | 2.6977 ± .0960 | .783 ± .044 | 74.6 ± 9.4 | 21.4 ± 2.3 | 22.6 ± 6.9 | 6.5 ± 1.2 |
| IB | 4 | 1.7280 ± .0050 | .668 ± .013 | 62.3 ± 3.7 | 24.1 ± 1.0 | 18.5 ± 2.1 | 7.0 ± .7 |
| | 2 | 2.7160 ± .0340 | .814 ± .052 | 48.8 ± .2 | 16.8 ± .8 | 22.3 ± 1.2 | 6.4 ± .1 |
| II | 7 | 2.4435 ± .0550 | .920 ± .013 | 51.0 ± 3.3 | 19.3 ± 1.2 | 23.0 ± 1.3 | 8.6 ± .6 |
| | 3 | 2.6619 ± .0850 | .752 ± .021 | 45.3 ± 3.1 | 12.7 ± .8 | 32.5 ± 2.4 | 8.9 ± .6 |
| III | 7 | 2.0150 ± .0230 | 1.090 ± .025 | 53.4 ± 1.6 | 28.9 ± 1.2 | 18.7 ± .7 | 10.2 ± .5 |
| | 3 | 2.0198 ± .0150 | .769 ± .010 | 54.1 ± 1.5 | 20.6 ± .7 | 26.2 ± 3.3 | 9.9 ± 1.2 |
| IV | 8 | 2.4250 ± .0850 | .903 ± .030 | 55.2 ± 3.1 | 20.7 ± .8 | 24.0 ± 1.6 | 9.0 ± .6 |
| | 8 | 2.3555 ± .0800 | .965 ± .100 | 53.0 ± 2.3 | 20.4 ± .6 | 24.6 ± 2.9 | 9.5 ± 1.1 |

*Experimental values underlined.

†Standard error.

have been increased somewhat. Losses of fat and nitrogen in the stools of 3 acidotic animals did not differ from those of 3 pair-fed controls. Increased adrenocortical secretion may also be responsible under the conditions of severe hypercapnia. The degree of anoxia (17 per cent oxygen in the ambient air) could not be sufficient to impose stress. Increased respiratory activity might increase caloric expenditure; however, voluntary activity was markedly diminished as compared with that of controls. Group III also clearly indicates that elevated chloride concentration or lowered bicarbonate concentration is not a critical alteration in growth failure associated with acidosis since bicarbonate concentration was markedly elevated and chloride concentration depressed in this group.

The most striking and unexpected finding was the absence of growth retardation in Group IV despite the presence of severe incompletely compensated hyperchloremic acidosis. This result shows clearly that acidosis does not invariably result in growth retardation. Why growth retardation did not occur is obscure. Acetazolamide does not lead to continued loss of fixed cation (sodium and potassium). The initial response to administration of carbonic anhydrase inhibitor is loss of sodium and potassium bicarbonate with rapid development of a hyperchloremic acidosis. Thereafter loss of fixed cation is no greater than in controls. Renal adjustment is relatively normal, although the sodium and the chloride ratio of tubular reabsorbate is set at a new level. Determination of the serum pH, carbon dioxide content, and chloride concentration of the serum at intervals throughout a 24-hour period did not demonstrate a temporary escape from acidosis that might possibly be expected because of the nocturnal eating habits of the rat which would lead to ingestion of acetazolamide for only a part of the day. Values given in Table I are representative of the levels at other hours although they were obtained in the morning.

The elevation of the serum inorganic phosphorus level may indicate a compensatory increase in secretion of anterior pituitary growth hormone.[18] However, since the glomerular filtration rate was not measured, a reduction of that function as a factor responsible for hyperphosphatemia cannot be ruled out. Calcification of the kidney was not observed. However, the calcium intake was lower than in the experiments of Harrison and Harrison.[19] Citrate excretion was not measured but was probably depressed. Similar growth studies in dogs[20] did not indicate

retardation; however, occasional spotty renal calcification was observed.

The results described in this paper have certain clinical correlates. West[21] has demonstrated that in chronic nephritis growth retardation had a high degree of correlation with the presence of metabolic acidosis. All patients with growth retardation had hyposthenuria, but many with loss of concentrating ability had normal growth. Cases of renal tubular acidosis exhibit severe growth failure which may be corrected completely or in part by administration of additional fixed cation in excess of anion (sodium and potassium bicarbonate, acetate, or citrate). Likewise, mild cases of renal tubular insufficiency may resemble the cases of Group I B in that growth retardation is observed accompanying the wasting of fixed cation in the absence of significant extracellular acidosis. Loading with ammonium chloride is necessary to establish the correct diagnosis in such cases.

No systematic observations of growth retardation have been recorded in uncomplicated respiratory acidosis. We have observed growth failure in isolated cases of the Pierre-Robin syndrome accompanied by respiratory acidosis. Acceleration of growth follows the gradual disappearance of carbon dioxide retention as respiratory obstruction diminishes.

The experiments reported here and clinical studies reported elsewhere suggest that the common denominator responsible for growth failure in acidosis or renal insufficiency may be the loss of fixed cation. The loss of the bulk cations (sodium, potassium, calcium, and magnesium) can be investigated by tissue or whole body analysis. Loss of trace cations represents a much more difficult investigative problem. Systematic study of growth patterns should be carried out in clinical states such as those indicated above, as well as in conditions such as the ketogenic diet and chronic acetazolamide administration in epileptic children.

## SUMMARY

Young male albino rats were subjected to the following procedures: Group I A, unilateral nephrectomy; Group I B, unilateral nephrectomy and 4 per cent ammonium chloride in the diet; Group II, 4 per cent ammonium chloride and 0.1 per cent acetazolamide in the diet; Group III, 13 per cent carbon dioxide in the ambient air; Group IV, 0.4 per cent acetazolamide in the diet.

Pair-fed controls were maintained under normal conditions. These various regimens produced acidosis which was hyperchloremic in Groups I B, I I, and I V and hypochloremic in Group III. In Groups I B, I I, and III, retardation of growth as manifested by weight gain and femur length was produced. This retardation in growth was not necessarily proportional to the degree of acidosis. In Group IV, in spite of severe hyperchloremic acidosis, no growth retardation was produced during the experimental period of 40 days.

The results of these experiments seem to indicate that acidosis may bear some relationship to retardation of growth. However, more problems are created than are solved by this work. Studies of hormone regulation of growth, tissue analyses, accurate studies of intake and loss of metabolites, and calorimetric studies of acidotic rats and patients all remain to be done in order to elucidate the basic mechanisms responsible for the retardation of growth produced in these experiments and observed in renal disease.

## REFERENCES

1. Wilkins, L.: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence, Springfield, Ill., 1957, Charles C Thomas, Publisher.
2. McCune, D. J.: Symposium on Pediatrics: Dwarfism, Clinic **2:** 380, 1943.
3. Talbot, N. B., Sobel, E. H., Burke, B. S., Lindermann, E., and Kaufman, S. B.: Dwarfism in Healthy Children: Its Possible Relation to Emotional, Nutritional, and Endocrine Disturbances, New England J. Med. **236**: 783, 1947.
4. Campbell, M., and Reynolds, G.: The Physical and Mental Development of Children With Congenital Heart Disease, Arch. Dis. Childhood **24:** 294, 1949.
5. Engle, Mary A., Holswade, G. R., Goldberg, H. P., and Glenn, F.: Present Problems Pertaining to Patency of Ductus Arteriosus. I. Persistence of Growth Retardation After Successful Surgery, Pediatrics **21:** 70, 1958.

6. Blodgett, F. M., et al.: Effects of Prolonged Cortisone Therapy on Statural Growth, Skeletal Maturation, and Metabolic Status of Children, New England J. Med. **254:** 636, 1956.
7. Wagner, Richard: Non-endocrine Dwarfism and Pseudoepiphyses, A. M. A. J. Dis. Child. **91:** 6, 1956.
8. Greene, C. H.: Bilateral Hypoplastic Cystic Kidneys. Report of Case Simulating Chronic Diffuse Nephritis in a Girl Three Years of Age, Am. J. Dis. Child. **24:** 1, 1922.
9. Albright, F., et al.: Metabolic Studies and Therapy in Case of Nephrocalcinosis With Rickets and Dwarfism, Bull. Johns Hopkins Hosp. **66:** 7, 1940.
10. Barber, H.: Renal Dwarfism, Quart. J. Med. **14:** 205, 1921.
11. Cooke, R. E., and Kleeman, C. R.: Distal Tubular Dysfunction With Renal Calcification, Yale J. Biol. & Med. **23:** 199, 1950.
12. Meneely, G. R., Tucker, R. G., and Darby, W. J.: Chronic Sodium Chloride Toxicity in the Albino Rat, J. Nutrition **48:** 489, 1952.
13. Addis, T., Mackey, E. M., and Mackey, L. L.: Effect on Kidney of Long Continued Administration of Diets Containing Excess of Certain Food Elements: Excess of Acid and Alkali, J. Biol. Chem. **71:** 157, 1926.
14. Jaffe, H. L., Bodanski, A., and Chandler, J. P.: Ammonium Chloride Decalcification as Modified by Calcium Intake: Relation Between Generalized Osteoporosis and Ostitis Fibrosa, J. Exper. Med. **56:** 823, 1932.
15. Burns, C. M.: The Effect of Continued Ingestion of Mineral Acid on Growth of Body and Bone and on Composition of Bone and of the Soft Tissues, Biochem. J. **23:** 860, 1929.
16. Bergstrom, W. H., and Wallace, W.: Bone as Sodium and Potassium Reservoir, J. Clin. Invest. **33:** 867, 1954.
17. Farquharson, R. F., Salter, W. T., Tibbets, D. M., and Aub, J. C.: Studies of Calcium and Phosphorus Metabolism: Effect of Ingestion of Acid-Producing Substances, J. Clin. Invest. **10:** 221, 1931.
18. Li, C. H., Geschwind, I., and Evans, H. M.: The Effect of Growth Hormone on the Inorganic Phosphorus Level in Plasma, Endocrinology **44:** 67, 1949.
19. Harrison, H. E., and Harrison, H. C.: Inhibition of Urine Citrate Excretion and Production of Renal Calcinosis in the Rat by Acetazoleamide (Diamox) Administration, J. Clin. Invest. **34:** 1662, 1955.
20. Maren, T. H., Mayer, E., and Wadsworth, B. C.: Carbonic Anhydrase Inhibition. I. The Pharmacology of Diamox 2-Acetylamino-1, 3,4-Thiadiazole-5-Sulfonamide, Bull. Johns Hopkins Hosp. **95:** 199, 1954.
21. West, C. D., and Smith, W. C.: Attempt to Elucidate Cause of Growth Retardation in Renal Disease, A. M. A. J. Dis. Child. **91:** 460, 1956.

# *A new autosomal trisomy syndrome: multiple congenital anomalies caused by an extra chromosome*

**David W. Smith, M.D.,* Klaus Patau, Ph.D., Eeva Therman, Ph.D., and Stanley L. Inhorn, M.D.**

MADISON, WIS.

THE normal human complement of chromosomes consists of 22 pairs of autosomes and 2 sex chromosomes. On the basis of their size and structure they can be classified into identifiable groups. The members of the 23 pairs are segregated during meiosis when the number of somatic chromosomes is reduced from 46 to 23, the number found in gametes. An accidental failure of this disjunction of pairs of chromosomes has been found to occur occasionally in many organisms and no doubt can happen also in man. In the latter, nondisjunction of one pair will result in a gametic chromosome number of 22 or 24. The combination of a gamete with 24 chromosomes with a normal one would produce a "trisomic" zygote in which one chromosome exists in triplicate rather than as a normal pair, the total number of chromosomes being 47. The presence of an extra chromosome amounts to an altered genetic balance which is almost bound to interfere with development in a variety of ways. This might result in death of the fetus or at least in a complex of congenital anomalies.

Until very recently it was unknown whether any human trisomics would be at all viable. If so, it was to be expected that individuals having the same extra chromosome, which means the same extra set of gene loci, would show sufficient similarity in their anomalies to be recognized as representatives of a specific syndrome. To date 3 such "trisomy syndromes," as Patau, Smith, Therman, Inhorn, and Wagner[1] called them, have been recognized. In one of them the extra chromosome is the X chromosome, in the other two an autosome.

Jacobs, Baikie, Court Brown, MacGregor, Maclean, and Harnden[2] found 3 instead of the normal 2 X chromosomes in a woman who had signs suggestive of an estrogen deficiency. Jacobs, Baikie, Court Brown, and Strong,[3] and Lejeune, Turpin, and Gautier[4]

*From the Departments of Pediatrics and Pathology, Medical School, University of Wisconsin, Madison, Wis.*

*This investigation was supported in part by Grant No. C-3313 and Grant No. A-3645 from the National Institutes of Health, Public Health Service, and an Institutional Grant from the American Cancer Society Inc.*

**Address, Department of Pediatrics, University Hospitals, 1300 University Avenue, Madison 6, Wis.*

showed that the syndrome of Mongolism is due to an extra chromosome which belongs to one of the 2 pairs of smallest autosomes. Patau and associates[1] described a complex of congenital anomalies in an infant who was found to be trisomic for one of the medium-sized autosomes. It can be expected that more individuals trisomic for this particular chromosome will become known and that they will display a similar clinical pattern. For this reason, this complex of anomalies will henceforth be referred to as a trisomy "syndrome." However, since the characterization is based on only 1 case, it is necessarily tentative. Patau and associates have also mentioned briefly 2 cases of another trisomy syndrome. These cases are the subject of the present communication.

**Fig. 1.** Infant (Case 8) at 2 months of age.

## CASE REPORTS

**Case 8.** *History.* The patient, a Caucasian girl, was born July, 1959, and was admitted at 2 months of age to the University Children's Hospital because of failure to gain weight. At the time of conception the mother was 46, the father was 49, and both were in good health. There are 14 living siblings ranging from 2 to 29 years of age, none of whom are known to have anomalies. The mother had 2 spontaneous abortions at the age of 41 years and 2 normal children since then.

A survey of the parental family trees disclosed a total of 12 aunts and uncles and 28 first cousins, none of whom is known to have a congenital anomaly.

The pregnancy was uncomplicated and lasted 7½ months. At birth the patient weighed 5 pounds 4 ounces. She was 17.5 inches long and was cyanotic.

*Physical examination.* At 2 months of age the patient was slim, dypsneic, sucked poorly, and had a weak cry. Her color was ashen, and she became cyanotic with crying. She was 19.5 inches long and weighed 5 pounds. There was a marked lack of subcutaneous fat. The circumference of the cranium was 14.0 inches and the anterior fontanelle was 3.0 by 4.0 cm. The skull could not be transilluminated. The ears were low set and the upper ears inclined in a posterior direction. The auricles were thin, and the right one looked elfinlike with a poorly formed scapha helix; the left was more ovaloid. Neither tragus was well developed, and anterior to the left ear were 2 pedunculated skin tags 0.5 and 0.7 cm. in length. The mandible was small. The hands appeared abnormal. The fingers were partially flexed and could be fully extended only with difficulty. The thumbs appeared small and were relatively narrow at the base. The fifth fingers were also small with no inturning of the distal phalanx. The wrists were partially flexed with a 15-degree ulnar deviation. The elbows, knees, and hips were partially flexed and it was difficult to extend them fully. There were symmetric deformities of the feet with convex soles and a 1 cm. posterior extension of the heels. This has been called "rocker bottom" feet.

The thoracic cage was wide in the anterior posterior diameter with a short sternum.

The heart was not enlarged as estimated by percussion. There was a palpable thrill at the left sternal border, and a Grade IV coarse systolic murmur was audible with maximal intensity in the third to fourth in-

terspace to the left of the sternum. The second pulmonic sound was loud and split. The systolic blood pressure by flush technique was 90 mm. Hg in the arms and 80 in the legs. The infant was dyspneic and occasional râles were audible at the posterior lung bases. The liver edge was palpable 2 cm. below the right costal margin.

The muscle tone in the extremities was increased to a degree suggesting spasticity. The Moro reflex was feebly present. A 1 cm. umbilical defect was present. No further anomalies were found. The external genitalia were normal.

*Laboratory data.* The urine was acid with a specific gravity of 1.007; no albumin, reducing substances, or cells were demonstrated. The hemoglobin was 8.4 Gm. per 100 ml. The serum urea nitrogen was 20 mg. and the fasting blood glucose was 50 mg. per 100 ml.

Roentgenographic examination of the skull showed poor ossification. Roentgenograms of the hand demonstrated a relatively small fifth metacarpal and small phalanges; those of the chest revealed increased pulmonary vascularity. The heart appeared slightly enlarged with an essentially normal contour.

*Hospital course.* The infant failed to thrive, took only 1 ounce of formula at a time, had cyanotic episodes with crying, and suddenly died on the ninth hospital day. She was then 2⅛ months of age. The patient did not vomit during the hospital stay.

*Findings at necropsy.* The heart was markedly enlarged, with moderate thickening of the wall of the right ventricle. A 1.0 cm. interventricular defect and a patent ductus arteriosus were found. The lungs revealed no gross anomaly but on microscopic examination showed moderate congestion and hypercellularity of the alveolar septa.

The pylorus was hard with a markedly thickened muscular wall. The pancreas appeared normal on gross evaluation. A section through the tail of the gland showed acinar and islet tissue within a dense fibrous network. The ducts were dilated. The appearance was considerably different from that ordinarily found in cystic fibrosis. Just proximal to the ligament of Treitz were 2 small nodules in the wall of the duodenum which were composed of well-formed acinar and islet tissue without fibrosis.

A rather large diverticulum of the ileum was located 76 cm. from the ileocecal valve. A horseshoe kidney was present with fusion of the lower poles over which the ureters passed from the normal-appearing pelvis. Microscopic examination revealed polymorphonuclear leukocyte infiltration within and around the renal tubules. The brain, bones, and joints were not investigated.

**Case 19.** The patient, a Caucasian boy, was born Oct. 5, 1959, and was seen at the Madison General Hospital where he had been admitted at 6 weeks of age because of multiple anomalies and failure to thrive.*

*History.* Each parent was 46 years of age at the time of conception of the patient. The mother was then suffering from apparent osteoarthritis of the spine. She became deaf following mastoiditis at 7½ years of age. The father has been deaf since he had spinal meningitis at the age of 14. There are 4 normal siblings, 2 boys and 2 girls, who range in age from 7 to 20 years. A survey of the parental family trees disclosed no known anomalies in 7 aunts and uncles, 17 first cousins, 20 great aunts and uncles, and 44 first cousins once removed. The pregnancy was said to be full term. On Jan. 9, 1959, close to the likely time of conception, the mother received diagnostic x-ray exposures during studies of the upper and lower gastrointestinal tract and of the dorsal spine. Fetal movement was less than had been experienced in previous pregnancies. The delivery was uncomplicated, and the baby weighed 5 pounds 1 ounce at birth. From birth the patient failed to thrive and had a poor dietary intake. Congenital heart disease with cardiac failure had been recognized.

*Physical examination.* At 2 months of age the infant showed constant dyspnea with dusky coloration which increased to cyanosis on crying; his sucking was poor, he was

*This patient was seen through the courtesy of Dr. Curtis R. Weatherhogg of Madison, Wis.

Fig. 2. Infant (Case 19) at 7 weeks of age.

lethargic and had a weak cry. He was 20.0 inches long and weighed 5 pounds 13 ounces. Subcutaneous fat was noticeable only in the buttocks and cheeks. The cranium was symmetrically small; the circumference was 13.0 inches. The ears were low set, and the scapha helix was incompletely developed and folded over with an acute angulation at the posterior border. The tragus was poorly developed. The mandible was symmetrically small. There were small inner epicanthic folds of the upper lid. The lens of the right eye contained a partial hazy white opacity. The optic discs appeared white. There was no retinal pigmentation.

The hands were abnormal. The fingers were held in a flexed position, and it was difficult to extend them fully. The third, fourth, and fifth fingers deviated in an ulnar direction in contrast to the position of the index finger. This gave rise to a V-shaped gap between the second and third fingers. The middle phalangeal joint of the index finger was partially fixed in extension. The palmar creases were anomalous, coming to a peak between the second and third fingers. In the feet there was partial syndactyly of the second and third toes.

The infant did not appear alert and did not follow objects with his eyes. A weak Moro reflex could be elicited. He was unable to support his head when held in the prone position. The extremities were maintained in a partially flexed position with increased resistance to passive flexion and extension. The deep-tendon reflexes were hyperactive.

The heart was enlarged to the left anterior axillary line as determined by percussion. There was a palpable thrill, and a Grade IV systolic murmur was audible over the entire precordium; it was loudest at the base in the third interspace to the left of the sternum. The pulmonary second sound was accentuated. The liver was palpable 3 cm. below the right costal margin.

A 1.5 cm. diastasis recti was palpable in addition to a 0.5 cm. umbilical defect. Bilateral inguinal hernias were present. The external genitalia were those of a normal male with testes in the scrotum.

*Laboratory data.* The hemoglobin was 13.1 Gm. per 100 ml. and the white blood count was 17,900 per cubic millimeter with 46 per cent segmented neutrophils, 2 per cent basophils, 1 per cent eosinophils, 36 per cent lymphocytes, and 15 per cent monocytes. The urinalysis showed an acid reaction; albumin, reducing substances, and cells were absent.

Roentgenographic examination of the chest showed enlargement of the heart both to the left and right and fullness of the pulmonary conus area. The pulmonary vascularity was increased. No abnormality was noted in the skull. An intravenous pyelogram appeared normal. The roentgenographic findings in the hands demonstrated no abnormality other than the anomalous position of the fingers as previously mentioned. There was a spina bifida of the ninth, tenth, and eleventh thoracic vertebrae.

*Skin patterns.* In an effort to exclude the possibility of Mongolism, skin patterns were studied.* A logarithmic index of –6.65 was

*The study of the skin patterns was conducted by Dr. Irene Uchida of The Children's Hospital, Winnipeg.

obtained. According to Walker[5] this is well within the normal range and entirely outside that of Mongoloids.

*Hospital course.* The evidences of cardiac failure continued until the infant died suddenly at 2 months of age.

*Findings at necropsy.* The heart was enlarged with a large right auricle and right ventricle; the wall of the latter was 8 mm. in thickness. There was a 0.5 cm. patency of the foramen ovale and a 1.0 cm. high interventricular septal defect. The ductus arteriosus was anatomically patent and small in caliber.

A 2 cm. diverticulum lined by intestinal mucosa was present 20 cm. above the ileocecal valve.

Microscopic sections of the lungs revealed a moderate amount of acute inflammatory cells in the walls of the bronchi, which also showed edema of the mucosa.

Gross and microscopic examinations of the brain, kidneys, adrenals, pancreas, liver, lymph nodes, and thymus demonstrated no abnormality.

## CHROMOSOME ANALYSIS

Chromosomes were studied in Feulgen slides made from cultures of bone marrow. The marrow was obtained by aspiration from a tibia of each patient. (For details of the culture technique, a modification of the procedure used by Ford, Jacobs, and Lajtha,[6] and for a description of the precautions taken to prevent subconscious bias from influencing the chromosome counts see Patau and others.[1])

The basic number of chromosomes of each patient was found to be 47 (Table I). The cell listed as having 46 chromosomes was a borderline case of acceptability for counting purposes and a miscount appears possible. In most cells there was no doubt about the number. There was at least one cell in the material from either patient which was clear enough to determine with confidence the number of chromosomes in each of the Groups A through G in the classification of Patau and associates. Apart from the presence of an extra chromosome, the complements of both patients appeared to be normal for their sex, which means in particular that the girl (Case 8) had 16 C chromosomes and 4 G chromosomes while the corresponding numbers in the boy (Case 19) were 15 and 5, C and G being the groups to which the X and the Y chromosomes belong, respectively. This, in conjunction with the results obtained in buccal smears (Case 8, chromatin-positive; Case 19, chromatin-negative), leaves no doubt that one (Case 8) had the sex chromosome constitution XX while the other had XY.

**Table I.** Chromosome counts in bone marrow cells

| *Patient* | *Chromosome number* | | | | | *Number of mitoses* |
|---|---|---|---|---|---|---|
| | *45* | *46* | *47* | *48* | *49* | |
| Female (Case 8) | – | – | 9 | – | – | 9 |
| Male (Case 19) | – | 1 | 6* | – | – | 7 |
| Total | – | 1 | 15 | – | – | 16 |

*This includes two oversquashed mitoses which had been counted only to obtain a lower limit of the chromosome number.

The extra chromosome in each patient belonged to the autosomal E group as this contained 7 chromosomes in contrast to the 3 pairs, No. 16, 17, and 18 (17 to 19 in the enumeration by Ford, Jacobs, and Lajtha[6]), which are characteristic for the normal complement. The chromosomes of these 3 pairs are appreciably larger than the autosomes of the 2 F and the 2 G pairs and not much smaller than the 3 pairs of acrocentric D chromosomes to which the extra chromosome belongs that is presumably responsible for the syndrome described by Patau and others. The 3 pairs of E chromosomes can be distinguished from each other, but none of the relatively few mitoses found in the present material showed all 7 E chromosomes with that optimal clarity which is required for a definite identification of the extra chromosome within the E group. As both patients died before more bone marrow could be collected, the question must be left open which of the E chromosomes, when present in triplicate, causes the syndrome described in this report.

**Table II.** Autosomal trisomy syndromes*

| Area of involvement and anomaly | | Description of syndrome: Present report, Case 8 | Description of syndrome: Present report, Case 19 | Description of syndrome: Patau and others[1] | Mongolism† (%) |
|---|---|---|---|---|---|
| Extra chromosome belonging to group‡ | | E | E | D | G |
| Neural system | Mental defect | P | P | + | 100 |
| | Deafness (neural ?) | – | – | + | – |
| | Spasticity | + | + | – | – |
| | Minor motor seizures | – | – | + | 2 |
| Neural or muscular system | Hypotonia | – | – | – | 66 |
| Hands | Flexion of fingers | + | + | – | – |
| | Ulnar deviation of third, fourth, fifth fingers | – | + | – | – |
| | Trigger thumbs | – | – | + | ? |
| | Inturned fifth fingers | – | – | – | 68 |
| | Horizontal palmar crease | – | – | + | 48 |
| Feet | Rocker bottom | + | – | – | – |
| | Syndactyly | – | + | – | – |
| | Polydactyly | – | – | + | – |
| | Wide gap first to second toe | – | – | – | 44 |
| Thoracic cage | Pidgeon breast | + | – | – | 14 |
| Vertebrae | Spina bifida occulta | ? | + | ? | ? |
| Cranium | Flat occiput | – | – | – | 82 |
| Mandible | Micrognathia | + | + | – | – |
| Auricle | Malformed | + | + | – | 48 |
| | Low set | + | + | – | Unusual |
| Eye | Inner epicanthic fold | – | + | – | 50 |
| | Cataract | – | + | – | – |
| | Apparent anophthalmia | – | – | + | – |
| | Speckled iris | – | – | – | 30 |
| Mouth | Cleft palate | – | – | + | – |
| | Harelip | – | – | + | – |
| Skin | Multiple hemangiomata | – | – | + | – |
| Abdominal wall | Umbilical hernia | + | + | – | ? |
| | Inguinal herniae | – | + | – | ? |
| | Diastasis recti | – | + | – | 76 |
| Heart | Unspecified anomaly | | | | 28 |
| | Interventricular defect | + | + | + | Common |
| | Patent ductus | + | + | – | ? |
| | Rotational anomaly | – | – | + | ? |
| Kidney | Horseshoe | + | – | ? | ? |
| Intestine | Diverticulum (Meckel's ?) | + | + | ? | ? |
| | Pyloric tumor | + | – | ? | ? |
| | Ectopic pancreatic tissue | + | – | ? | ? |
| Maternal age at conception (years) | | 46 | 45 | 25 | Over 40 24% |

†Frequencies as found in random sample of 50 Mongoloids analyzed by Levinson, Friedman, and Stamps.[7]

‡This classification was proposed by Patau and associates.[1]

*+, anomaly present; P, probably present; —, absent; ? uncertain.

## DISCUSSION

The similarity in the clinical pattern of the anomalies in the present 2 cases leaves little doubt that the extra chromosome is the same in each, even though the chromosome analysis revealed only that it belongs to the E group in the classification of Patau and associates. The clinical findings, in conjunction with the type of the extra chromosome, distinguishes these cases from both Mongolism and the case of trisomy described by Patau (Table II).

The most striking anomalies in the present patients are the small mandible and the low-set malformed ears, indicating incomplete development of the hypomandibular arch. Potter[8] reported the very same type of anomalous development in 20 patients with bilateral renal agenesis, 4 of whom were afflicted with contractures of hips and elbows and 7 of whom had clubfeet. The girl (Case 8) in this report had an anomaly of the kidney in addition to flexion deformities and clubfeet. This invites the speculation that some instances of renal agenesis with low-set ears might possibly be examples of the present trisomy syndrome, but with a more severe manifestation of the renal defect.

In the girl (Case 8) and to a lesser extent in the boy (Case 19) the anomalies of joint mobility, while not conforming with the classical picture, were suggestive of arthrogryposis multiplex congenita. This abnormality was claimed by Middleton[9] to result from a failure of normal development of certain muscles with subsequent degenerative changes in these muscles leading to secondary joint fixation. However, the basic cause of this disease remains unknown. A 5-month-old white boy, recently admitted to the University Children's Hospital, exhibited a classical picture of arthrogryposis multiplex congenita as described by Stern.[10] The number of chromosomes in the marrow cells of this patient was 46, suggesting that this disease is not generally caused by an extra chromosome.

The two patients appeared to be mentally defective, but this cannot be stated with certainty, as they had cardiac failure at the time of examination and died at 2 and 2½ months of age, respectively. Both infants were developmentally retarded with signs of spasticity. If, in the future, cases surviving the period of early infancy should be found, the question of mental defect could be solved. In view of the complexity of cerebral structure and function the presence of mental deficiency in the two known autosomal trisomy syndromes is not surprising (Table II). Quite likely a mental defect will also be a frequent or even ubiquitous feature in the present syndrome.

The occurrence of an extra chromosome is presumably always the result of nondisjunction, which is essentially a sporadic accident. There is no history of similarly affected infants or of other major congenital anomalies in the families of either of the present patients. The recurrence of Mongolism in the same family is rare; Benda,[11] for instance, found it twice in 255 families. The increase in frequency of Mongolism with maternal age indicates that nondisjunction is more frequent in older women. It is, therefore, not surprising that the

**Fig. 3.** Top: 47 chromosomes in bone marrow cell of an infant (Case 41) trisomic for chromosome No. 18; original magnification × 2,840. Bottom: the E chromosomes from the same cell; original magnification × 4,030.

mothers of both patients were of advanced age at the time of conception (Table II). The mother of one (Case 19) also received diagnostic x-ray exposure close to the presumed time of conception. Whether this had any etiologic significance in the present case is a question which must remain open.

In Mongolism a multiplicity of anomalies are seen but, with the exception of mental retardation, there is not one which is found in all Mongoloid patients. However, the pattern of anomalies is usually sufficiently characteristic to permit a clinical diagnosis. In the instance of the present syndrome the situation is likely to be similar. As more cases are identified, the range of anomalies can be established. The most regularly-occurring ones will then form a pattern upon which the diagnosis can be based. The individual anomalies, which characterize Mongolism when they occur together, are occasionally found as isolated features in non-Mongoloids. Similarly, all the component anomalies of the present syndrome probably also occur as single or even multiple defects in individuals having the normal complement of chromosomes. Such occurrences can come about by mutation or, perhaps, by a non-genetic prenatal accident.

The true incidence of the present syndrome is unknown. Nondisjunction of the responsible chromosome may not be as frequent as in the case of Mongolism. Furthermore, the severe nature of the anomalies displayed by the 2 patients suggests that fetal death, stillbirth, or death in early infancy may lower the observed frequency of the syndrome.

## SUMMARY

Two patients are described who died in early infancy. Each displayed similar complexes of congenital anomalies of which the following ones were found in both: low-set and malformed ears, small mandible, flexion deformities of the fingers, anomalous feet, interventricular septal defect, spasticity with probable mental defect, and diverticulum of the intestine.

The chromosome number was 47, the extra chromosome appearing to be the same one in each instance; it belongs to the E group in the classification of Patau and associates.[1]

The mothers of both patients were of advanced age at the time of conception.

## ADDENDUM

Since the submission of the present report, 4 more infants, all unrelated, with the same syndrome have been discovered by the authors. All have 47 chromosomes as illustrated, for one of them, in Fig. 3. The extra chromosome is No. 18, which is also its number in the classification by Ford and colleagues.[6] Recently Edwards and colleagues[12] have described a trisomy syndrome that seems to be the same as the one presented above. However, these authors identify the extra chromosome as No. 17.

## REFERENCES

1. Patau, K., Smith, D. W., Therman, E., Inhorn, S. L., and Wagner, H. P.: Multiple Congenital Anomaly Caused by an Extra Autosome, Lancet **1:** 790, 1960.
2. Jacobs, P. A., Baikie, A. G., Court Brown, W. M., MacGregor, T. N., Maclean, N., and Harnden, D. G.: Evidence for the Existence of the Human "Super Female," Lancet **2:** 423, 1959.
3. Jacobs, P. A., Baikie, A. G., Court Brown, W. M., and Strong, J. A.: The Somatic Chromosomes in Mongolism, Lancet **1:** 710, 1959.
4. Lejeune, J., Turpin, R., and Gautier, M.: Le Mongolisme: premier exemple d'aberration autosomique humaine, Ann. génét. **1:** 41, 1959.
5. Walker, N. F.: The Use of Dermal Configurations in the Diagnosis of Mongolism, J. PEDIAT. **50:** 19, 1957.
6. Ford, C. E., Jacobs, P. A., and Lajtha, L. G.: Human Somatic Chromosomes, Nature **181:** 1565, 1958.
7. Levinson, A., Friedman, A., and Stamps, F.: Variability in Mongolism, Pediatrics **16:** 43, 1955.
8. Potter, E. L.: Bilateral Renal Agenesis, J. PEDIAT. **29:** 68, 1946.
9. Middleton, D. S.: Studies on Prenatal Lesions of Striated Muscle as a Cause of Congenital Deformity, Edinburgh M. J. **41:** 401, 1934.
10. Stern, W.: Arthrogryposis Multiplex Congenita, J. A. M. A. **81:** 1507, 1923.
11. Benda, C. E.: Mongolism and Cretinism, ed. 2, New York, 1949, Grune & Stratton, Inc.
12. Edwards, J. H., Harnden, D. G., Cameron, A. H., Crosse, V. M., and Wolff, O. H.: A New Trisomic Syndrome, Lancet **1:** 787, 1960.

# *Amino acid and alpha-keto acid-induced hyperinsulinism in the leucine-sensitive type of infantile and childhood hypoglycemia*

**Melvin M. Grumbach, M.D.,* and Selna L. Kaplan, M.D.****

NEW YORK, N. Y.

"SPONTANEOUS" hypoglycemia may result in irreversible cerebral and lower motor neuron damage.[1-8] To minimize such unfortunate consequences it is important that the varied neurological manifestations of hypoglycemia and the variety of disorders associated with this clinical abnormality be more widely appreciated. The clinical manifestations and the causes of hypoglycemia[2, 3, 5, 9-22] have been extensively reviewed in recent reports. An important though poorly understood syndrome associated with severe hypoglycemia in children is "idiopathic" infantile hypoglycemia as defined by McQuarrie,[5] which in some instances is effectively managed by treatment with adrenocorticotropin or corticosteroids. In 1956, Cochrane, Payne, Simpkiss, and Woolf[23] reported that administration of leucine induced a fall in the concentration of blood sugar in some patients with this syndrome.

The present report describes studies on 2 patients with the leucine-sensitive type of "idiopathic" hypoglycemia. The results indicate that L-isoleucine and the α-keto acid analogue of L-leucine, α-ketoisocaproic acid, as well as L-leucine, induce hypoglycemia by increasing the concentration of plasma insulin. This effect seems to result from their action on the mechanism of insulin secretion.

*From the Department of Pediatrics, College of Physicians and Surgeons, Columbia University and the Babies Hospital, New York, N. Y.*

*This work was supported by a grant from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.*

*Presented in part at the annual meeting of the American Society for Clinical Investigation, Metabolism Section, May 1, 1960, Atlantic City, N. J.*

**Address, The Babies Hospital, Columbia-Presbyterian Medical Center, 622 West 168th Street, New York 32, N. Y.*

***Postdoctoral Fellow, National Institutes of Health, and Fellow in Pediatric Endocrinology, The Babies Hospital, New York, N. Y.*

## CASE HISTORIES

**C. D.** (No. 140-96-94). The patient was admitted to Babies Hospital at 15 months of age in a semi-stuporous state. He was the only child of a 19-year-old mother and 18-year-old father. There was no history of consanguinity and the family history was negative for endocrine diseases. A maternal aunt had a convulsive disorder of unknown etiology.

His early infancy was uneventful. It had been noted, however, that his motor development was not commensurate with his age.

His mother became aware of an unusual pattern of behavior at 8 months of age. Each morning, about one hour after a breakfast of eggs, bacon, cereal, juice, and milk, he became drowsy and weak. During these periods, he would lie on the floor responding only to strong stimulation. No clonic or tonic movements were noted. At first the episodes of altered consciousness lasted 5 to 10 minutes and occurred once a week, but gradually they increased in both frequency and duration. During the 2 months prior to admission, periods of drowsiness lasted as long as 6 to 8 hours. On two occasions, these "spells" terminated in a generalized seizure.

On admission at 2 P.M. the patient was found to be flaccid, with eyes staring straight ahead; he responded slightly to strong stimulation. His pulse rate was 120; the blood pressure was 98 mm. Hg systolic and 65 diastolic. He weighed 9,230 grams; his length was 81 cm.; head circumference, 46.5 cm.; and chest circumference, 44 cm. (all within 1 standard deviation of the mean value for his age). Physical examination was otherwise unremarkable. A sample of blood was drawn for glucose analysis and the child was given a glucose feeding, to which he responded promptly. Over a period of 30 minutes he became alert and playful. The blood sugar concentration on admission was 25 mg. per 100 ml.

*Laboratory Studies.* A hemogram showed a hemoglobin of 9.8 Gm. per 100 ml., a red blood cell count of 4.86 million per cubic millimeter, and a normal leukocyte count and differential. A sickle cell preparation was negative. The urine had a specific gravity of 1.018, pH of 4.5, and gave a negative reaction for sugar, protein, and acetone. The formed elements were unremarkable. The serum calcium content was 10.9 mg. per 100 ml., nonprotein nitrogen 24 mg. per 100 ml., sodium 140.6 mEq. per liter, potassium 4.6 mEq. per liter, chloride 100 mEq. per liter, and carbon dioxide combining power 22.4 mEq. per liter. The cephalin flocculation and thymol turbidity tests were negative.

The zinc turbidity was 16.1 units, serum albumin 4.7 Gm. per 100 ml., serum globulin 2.5 Gm. per 100 ml., cholesterol 174 mg. per 100 ml., serum glutamic oxalacetic transaminase 35 units, and serum glutamic pyruvic transaminase 18 units. The excretion of urinary 17-ketosteroids was 0.2 to 0.8 mg. per day, and of 17-ketogenic steroids, 4.5 to 4.7 mg. per day. The excretion of catechol amines, determined by a modification of the procedure of Von Euler and Floding,[24a] was normal (epinephrine 1.2 μg per day, norepinephrine 8 μg per day).

Roentgenograms of the skull were normal; the bone age was commensurate with his chronologic age. An electroencephalogram showed a normal sleeping record.

Several fasting venous blood sugar determinations gave values of 40 to 62 mg. per 100 ml., whereas capillary blood sugar varied from 27 to 35 mg. The concentration of venous blood sugar 1½ hours after lunch was 60 mg. per 100 ml.

Various carbohydrate and amino acid tolerance tests were performed which indicated a hypoglycemic response to the administration of leucine. Subsequently, the patient was started on supplemental glucose feedings 30 minutes after meals. Since discharge, there has been a marked change in the daily behavior of this boy to that of a normal hyperactive 2-year-old. By the time of his second admission he had learned to walk and to speak a few simple words. He became both inventive and mischievous and was now quite reluctant to take an afternoon nap.

Psychometric examination was restricted by limited verbal ability, but no perceptual or motor retardation was detected.

**T. O.** (No. 30-04-67). A 30-month-old boy was admitted to Babies Hospital for evaluation of recurrent seizures and hypoglycemia. He is the youngest of 7 children of a 37-year-old mother and a 38-year-old father. There was a strong family history of diabetes in both the paternal and maternal lines. No evidence of convulsive tendencies or mental disturbances was obtained. However, one female sibling, aged 16 years, was known to have infrequent fainting spells and the mother has had periods of weakness in mid-morning and mid-afternoon.

The first manifestations of illness became apparent at 4 months of age when the parents noted daily irregular jerking movements of the extremities without clonic or tonic movements as well as occasional grand mal seizures. Subsequently, these daily episodes merged into a prolonged semicomatose state which began soon after breakfast or any high-protein meal and lasted for several hours. At times they were associated with sweating, coldness, and pallor.

At 6 months of age, he was admitted to a local hospital. The fasting blood sugar was 70 mg. per 100 ml., serum calcium 9.4 mg. per 100 ml., and serum phosphorus 5.3 mg. per 100 ml. Spinal tap showed clear fluid under no increased pressure. The protein and chloride content of the fluid was normal; the concentration of sugar was 22 mg. per 100 ml. Only a small amount of clear fluid was obtained from bilateral aspirations of the subdural space.

He was referred to the Seizure Clinic at Babies Hospital for diagnosis and therapy at the age of 11 months. Preliminary work-up showed fasting blood sugar determinations of 42 to 50 mg. per 100 ml. Roentgenograms of skull and an electroencephalogram were reported to be normal for his age. At 1 year of age he was admitted to the Child Neurology Service at Babies Hospital for appraisal of his convulsive status. During his hospital stay he was noted to be continuously lethargic. The glucose and Adrenalin tolerance tests were normal. On two occasions the fasting blood sugar levels were 37 and 46 mg. per 100 ml., whereas at other times the fasting values varied from 55 to 75 mg. per 100 ml. The serum calcium concentration was 11.5 mg. per 100 ml., serum phosphorus 4.7 mg. per 100 ml., and cholesterol 140 mg. per 100 ml., liver function tests were normal.

He was discharged on anticonvulsant therapy and the parents were advised to give him additional sugar feedings. Although it soon became apparent that the medication did not decrease the frequency of these episodes the patient did not return to the clinic for 2 years. The mother now described periods in which the patient was weak and drowsy; the latter lasted from 5 minutes to many hours. An electroencephalogram showed evidence of an abnormal pattern consistent with brain damage. He was readmitted to Babies Hospital at 40 months of age for re-evaluation of his hypoglycemia and convulsive episodes. A definite correlation between the occurrence of seizures or episodes of altered consciousness and meals composed of foods with a high leucine content was described by the parents.

A L-leucine tolerance test demonstrated a hypoglycemic response. Postprandial glucose feedings were initiated but a change in his behavior was not evident before discharge.

The parents were advised to continue the anticonvulsant therapy in addition to supplemental feedings of sugar after each protein meal. They reported that it was necessary to give him orange juice within 15 minutes, particularly after eggs and tuna fish salad, and to offer an additional supplement within the hour. They voluntarily discontinued the anticonvulsant therapy while increasing the glucose feedings.

Since discharge, his mental alertness and activity have improved. There has been no recurrence of episodes of drowsiness. However, he continues to show signs of brain damage and delayed mental development.

## METHODS

The concentration of blood glucose was determined by the glucose oxidase procedure,[24] blood sugar by the micro method of Benedict including the addition of bisulfite,[25] and plasma lactate by the method of Barker and Summerson.[26] Serum inorganic phosphate was estimated by the method of Fiske and SubbaRow.[27]

Plasma amino acids were analyzed by the column chromatographic procedure of Moore and Stein.[28] Amino acids in urine were detected and measured semiquantitatively by a modification of the method of Dent[29]; total urinary α-amino nitrogen was determined by the procedure of Van Slyke and co-workers.[30]

Urinary α-keto acids were determined semiquantitatively following conversion to 2,4-dinitrophenyl hydrazone derivatives, extraction with solvent, and resolution by paper chromatography according to the method of Menkes.[31] Urinary neutral 17-ketosteroids were measured by a modification of the method of Callow, Callow, and Emmens, the Zimmerman reaction being applied according to Wilson.[32] Urinary 17-ketogenic steroids were determined by the method of Birke, Diczfalusy, and Plantin.[33]

For the determination of plasma insulin, samples of venous blood were drawn into syringes wetted with a solution of heparin. Specimens were chilled in an ice bath and centrifuged at 4° C. The plasma supernate was removed and stored at −20° C. The concentration of insulin in thawed specimens was determined by the immunoassay method of Yalow and Berson.[34, 35]

Purified L, D, and DL amino acids* and α-keto acids† were administered as solutions or suspensions by stomach tube in the young patients and by mouth in older subjects. α-Keto acids were prepared for intravenous infusion by the addition of physiologic salt solution and a sufficient amount of 10 per cent $Na_2CO_3$ (w/v) to bring the pH to neutrality, following which the solution was filtered through a millipore filter.

## RESULTS

**Tolerance tests.** Comparable results were obtained from the analyses of duplicate specimens of blood for sugar by the method of Benedict[25] and for glucose by the glucose oxidase procedure[24] (Fig. 1). Glucose, epinephrine, and glucagon tolerance tests in patients C. D. and T. O. showed, in general, low concentrations of blood sugar or glucose in fasting specimens but a normal-shaped curve.

**Amino acid.** The effect on the concentration of blood glucose of the administration of L-leucine (150 mg. per kilogram) by stomach tube was rapid and dramatic (Fig. 2). Within 15 minutes the sugar content diminished and signs supervened which simulated the altered state of consciousness observed in these patients following a meal high in protein composition. Usually, the nadir of the blood sugar curve was reached at 30 minutes, following which time the concentration of blood sugar failed to increase appreciably for 60 minutes. No glucose was detected in the urine during the course of these experiments. For comparison blood glucose values obtained during a control period at the same time of day are plotted. On one occasion early in the study, the hypoglycemia induced in patient C.D. was associated with involuntary movements of the extremities; the latter were quickly terminated by prompt administration of a glucose solution intravenously. To minimize this

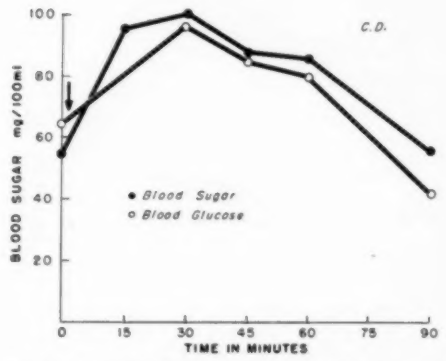



**Fig. 1.** A comparison of capillary blood sugar as determined by the glucose oxidase (open circles) and by the Benedict micro (dots) method.

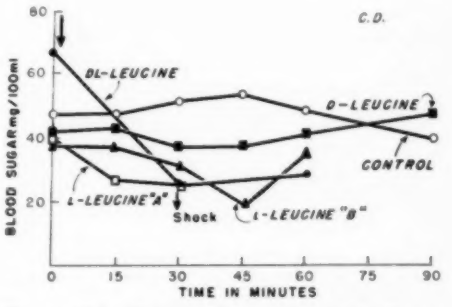



**Fig. 2.** The effect on blood sugar of feeding L-leucine ("*A*" = 150 mg. per kilogram, "*B*" = 50 mg. per kilogram) and D-leucine and DL-leucine (150 mg. per kilogram) to patient C. D.

*Obtained from K & K Laboratories, Mann Research Laboratories, and Eastman Kodak Co.

†Obtained from K & K Laboratories.

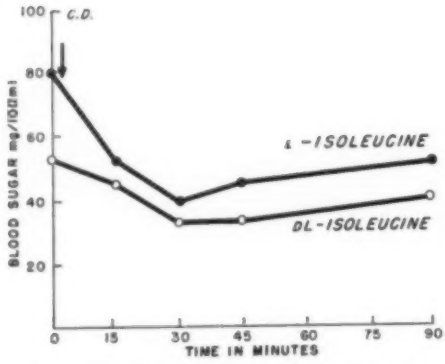



**Fig. 3*A*.** The effect of L-isoleucine and DL-isoleucine (150 mg. per kilogram) on blood sugar in patient C. D.

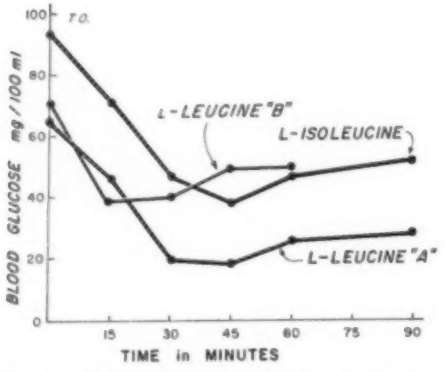



**Fig. 3*B*.** The effect on the blood sugar of L-isoleucine (150 mg. per kilogram) and L-leucine ("*A*" = 150 mg. per kilogram; "*B*" = 50 mg. per kilogram in patient T. O.

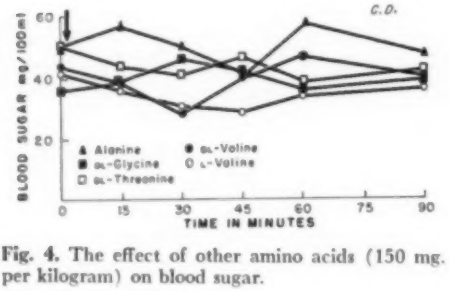



**Fig. 4.** The effect of other amino acids (150 mg. per kilogram) on blood sugar.

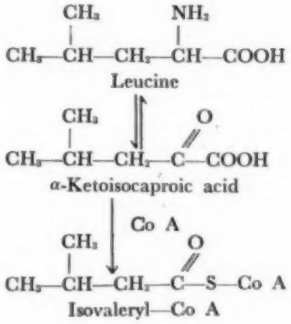
untoward reaction, subsequent tests usually were performed 2 hours after the administration of orange juice fortified with cane sugar at 6 or 7 A.M.

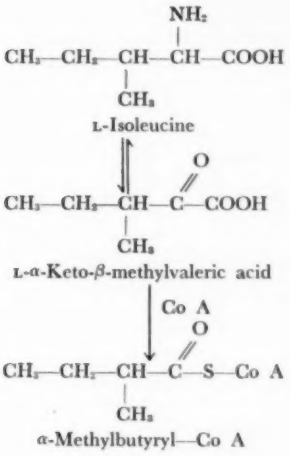
As shown in Fig. 2, administration of the D-isomer of leucine had no effect on the blood sugar. Since feeding the same amount (150 mg. per kilogram) of racemic leucine (DL) led to a fall in blood sugar content comparable to that obtained with L-leucine, it seemed likely that a dose of 75 mg. per kilogram (or less) of L-leucine would be sufficient to elicit a hypoglycemic effect in these patients. It was shown subsequently that a definite response could be obtained by feeding 50 mg. per kilogram of L-leucine (Fig. 2).

The possibility was next considered that other amino acids similar in structure to leucine might exhibit a hypoglycemic action in these individuals. Leucine is one of three branched-chain amino acids; the others are isoleucine and valine. All three are members of the group of aliphatic amino acids with a monoamino-monocarboxyl structure. Figs. 3*A* and 3*B* show the blood sugar response to L- and DL-isoleucine in patients C. D. and T. O., respectively. A fall in the concentration of blood glucose similar to, but perhaps slightly less, than that demonstrated for L-leucine was obtained. In addition the patients were drowsy and, when aroused, irritable. It is of interest that leucine is a ketogenic but not glycogenic amino acid, whereas isoleucine is both ketogenic and glycogenic.

Valine, a glycogenic amino acid, showed an equivocal effect which was insufficient to be considered clearly significant. L-alanine and L-glycine, other amino acids with a monoamino-monocarboxyl structure, did not affect the blood sugar content, nor did the administration of DL-threonine, a hydroxy-amino acid (Fig. 4). No neurological symptoms were observed following their administration.

**Alpha-keto acid.** Leucine, isoleucine, and valine are rapidly degraded by a similar sequence of reactions[38-39] which have been characterized in animal tissues mainly by Coon and associates.[36] The first two steps involve (1) reversible enzymatic transamination to the α-keto acid analogue and (2) irreversible oxidative decarboxylation to yield the acyl-coenzyme A derivative of the α-keto acid indicated:




The following studies were performed to determine whether administration of the α-keto acid metabolites of the above amino acids elicited hypoglycemia. The α-keto acid analogue of leucine, α-ketoisocaproic acid, when given by stomach tube to patient C. D. in a dose two-thirds that of L-leucine induced a relatively small and delayed fall in the concentration of blood glucose (Fig. 5). It is known that this keto acid is a relatively labile substance. Because of the possibility that the molecule might undergo modification in the gastrointestinal tract or be slowly or poorly absorbed, it was administered intravenously as the sodium salt to patients C. D. and T. O. The sharp decreases in the concentration of blood glucose obtained in each patient with sodium α-ketoisocaproate are plotted in Fig. 5. The blood sugar response simulated the curves obtained with leucine and isoleucine. Independently, Mabry and collaborators[40] have observed a similar effect of the keto acid analogue of leucine in a patient with idiopathic hypoglycemia. A highly purified preparation of the keto acid analogue of isoleucine, L-α-keto-β-methylvaleric acid could not be obtained for study.

Evidence derived from experiments employing isotope tracers suggests that the keto acid analogue of leucine is decarboxylated to yield isovaleryl-coenzyme A.[36] Neither oral administration of isovaleric acid nor the intravenous injection of sodium isovalerate had a significant hypoglycemic effect (Fig. 6). These results are contrary to those reported previously by Cochrane and co-workers,[23] but the preparation of commercial isovaleric acid which they used was 60 per cent pure and may have been contaminated with other substances capable of lowering the blood-sugar concentration.

The intravenous injection of sodium α-ketoisovalerate, the keto acid analogue of valine, had no demonstrable lowering effect on the blood glucose level (Fig. 6).

**Plasma and Urinary Amino Acids and Urinary α-Keto Acids.** To exclude the possibility that an abnormal accumulation of amino acids or α-keto acids in the blood had a role in the hypoglycemic response, the plasma amino acids and the urinary amino acids and α-keto acids were examined. Table I contains an analysis of plasma amino acids prior to and one hour after the administration of leucine. The fasting pattern was normal and the changes in amino acid composition detected following a leucine feeding did not seem to differ significantly from those described previously by Snyderman and her associates[41] in 2 normal adults except for the fact that the concentration of tyrosine did not decrease in patient C. D. The general fall in plasma amino acid content may reflect, in part, interference with reabsorption by the kidney related to the leucine load and a secondary insulin-like effect on amino acid

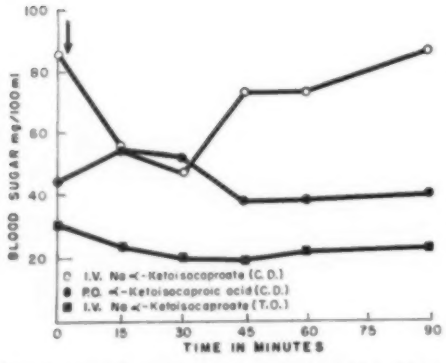



**Fig. 5.** The effect of α-ketoisocaproic acid on blood glucose (open circles and dots, patient C. D. 100 mg. per kilogram; solid squares, patient T. O. 75 mg. per kilogram intravenously).

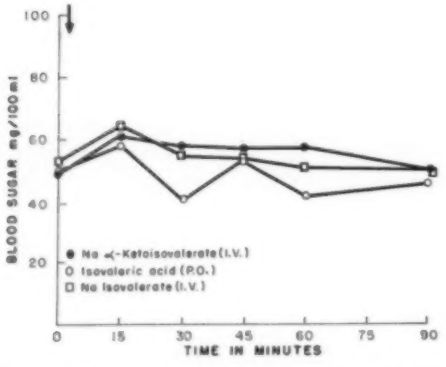



**Fig. 6.** The response of blood sugar to various keto acids (150 mg. per kilogram by mouth; 100 mg. per kilogram intravenously).

metabolism,[42] but the precise mechanism is obscure. It is of interest that the leucine content did not show an extraordinary rise, a result consistent with that previously obtained with a different method.[23] For example, the level of plasma leucine one hour after the administration of 150 mg. per kilogram of leucine is only about one-third the fasting level reported in a patient with maple syrup disease.[43] The increase in urea is small; it may represent increased amino acid metabolism.

The excretion of urinary amino nitrogen and the pattern of urinary amino acids were unremarkable. The urinary nitrogen in a 24-hour specimen was 2.35 Gm., of which 11.0 mg. (0.47 per cent) was α-amino nitrogen. No increase in leucine or isoleucine was detected in several samples of urine.

Keto acids were present in normal amounts in the urine (Table II). An abnormal accumulation of α-ketoisocaproic acid was not found after a leucine load; none of the serial samples of urine obtained during the subsequent 24 hours contained more than 400 μg per 100 ml.

The studies described above indicated that there was not an excess accumulation of amino acids in the blood or urine or of α-keto acids in the urine and strongly suggested that the hypoglycemia was not related to a metabolic block in the metabolism of the branched-chain amino acids or of their keto acid metabolites.

## STUDIES TO DEFINE THE MECHANISM OF HYPOGLYCEMIA

A hypoglycemic agent must exert its effect, either directly or indirectly, by (1) increasing glucose utilization or disposal, by (2) decreasing glucose production, or by (3) affecting both processes. The following series of experiments were designed to clarify the mechanism by which the administration of leucine, isoleucine, and their α-keto acid analogues might promote hypoglycemia in these patients.

**Effect of leucine and epinephrine on blood glucose.** The concurrent administration of leucine by gastric tube and of epinephrine subcutaneously to patient C. D. led to a rise in the level of blood glucose at 30 minutes and masked the effect of leucine (Fig. 7). The increase in the concentration of glucose although significant was less than that obtained with the same dose of epinephrine alone. These results suggested that glycogen depletion was not the major mechanism of action of leucine but did not exclude the possibility that leucine might inhibit hepatic glucose production. A moderate increase in plasma lactic acid content was noted, an effect consistent with increased glucose utilization.

**Effect of leucine on oxidation of glucose by whole blood.** In vitro studies of the effect of leucine on the oxidation of glucose to car-

bon dioxide by whole blood, carried out in collaboration with Dr. Paul A. Marks,[44] failed to demonstrate a direct action on glycolysis (Table III). The concentration of leucine used was approximately ten times the plasma concentration detected in patient C. D. one hour after a leucine load. No inhibition of phosphorylation, enhanced glucose utilization, or inhibition of the uptake of glucose by red blood cells was found.

A deficiency of glucose 6-phosphate dehydrogenase activity was demonstrated in the red blood cells of C. D. as well as in his mother's but this appeared to be an unrelated finding; this enzymatic defect was not found in T. O.

**Effect of leucine on lactate and phosphate metabolism and on arteriovenous sugar differences.** Following administration of leucine, patient C. D. showed a rise in the plasma lactate level and a fall in the plasma inorganic phosphate (Fig. 8). The maximum decrease in phosphate content occurred at 45 minutes during the nadir in the blood glucose curve, and the highest value obtained for lactate was detected at 60 minutes. A slight decrease in plasma potassium concentration was noted at 15 minutes; however, insufficient material was available for the measurement of potassium in blood specimens collected at 30 and 45 minutes. The results suggested increased peripheral utilization of glucose and were consistent with an insulin-like effect.[45-48] It is noteworthy that at no time was glucose detected in the urine.

Table IV shows capillary-venous and arteriovenous sugar differences in a limb after the administration of leucine. A significant increase in these differences was not found in specimens obtained 30 minutes after a leucine feeding. These observations at first hand seem contrary to the results one might expect if leucine had a direct or indirect insulin-like action. Apart, however, from technical considerations or of certain conditions in which the injection of a small amount of insulin may not cause a detectable increase in peripheral arteriovenous glucose differences,[49, 50] Madison and Unger[50, 51] have demonstrated differences in the effect of insulin injected into a peripheral vessel and directly into the portal circulation, the latter simulating the immediate action of endogenous insulin on hepatic glucose metabolism. The negative values obtained in the studies on patient C. D. for the ratio capillary or arterial glucose minus venous glucose/capillary or arterial glucose at 30 minutes suggest that the immediate fall in blood sugar concentration after a leucine feeding

**Table I.** Plasma amino acid (milligrams per 100 ml.) in patient C. D.

| *Amino acid* | *Fasting pre-leucine* | *1 Hour after L-leucine (150 mg./Kg.)* |
|---|---|---|
| Leucine | 1.19 | 4.55 |
| Isoleucine | 0.66 | 0.53 |
| Carboxymethyl cystine | 0.19 | 0.17 |
| Taurine | 0.45 | 0.56 |
| Threonine | 1.08 | 0.68 |
| Serine | 0.94 | 0.64 |
| Proline | 1.48 | 1.10 |
| Glutamic | 0.91 | 1.13 |
| Citrulline | 0.20 | 0.32 |
| Glycine | 1.20 | 0.83 |
| Alanine | 2.50 | 1.51 |
| α-Amino-α-butyric | 0.27 | 0.20 |
| Valine | 2.44 | 1.69 |
| ½ Cystine | 1.12 | 1.10 |
| Methionine | 0.21 | 0.33 |
| Tyrosine | 1.24 | 1.35 |
| Phenylalanine | 0.72 | 0.61 |
| Ornithine | 0.36 | 0.66 |
| Ethanolamine | 0.08 | 1.0 |
| Histidine | 0.90 | 1.34 |
| 1 Methyl histidine | Trace | 0.53 |
| 3 Methyl histidine | Trace | 0.31 |
| Lysine | 1.58 | 2.81 |
| Tryptophane | Trace | 0.50 |
| Arginine | 0.91 | 1.31 |
| Urea | 28.1 | 31.9 |

**Table II.** Urinary keto acids* (semiquantitative estimation)

| | *mg./100 ml.* | *mg./day* |
|---|---|---|
| Pyruvate | 4 | 15 |
| α-Ketoisocaproic acid | 0.5 | 1.9 |
| α-Ketoglutaric acid | 4 | 15 |
| α-Ketoisovaleric acid | Trace | |
| Acetoacetic acid | 1+ | |
| Succinic semialdehyde | Trace | |

*Following a leucine feeding none of serial samples of urine contained more than 0.4 mg. per 100 ml. of α-ketoisocaproic acid. The pattern of keto acids was not abnormal in any of the specimens analyzed.

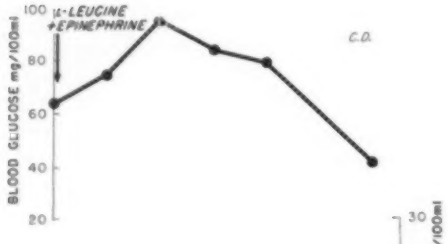



**Fig. 7.** The effect of simultaneous administration of epinephrine (0.2 mg. subcutaneously) and L-leucine (150 mg. per kilogram by mouth on blood glucose and plasma lactate.

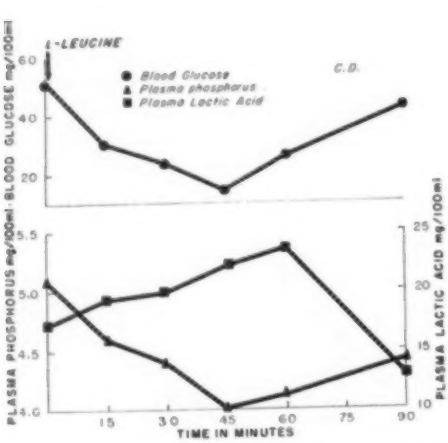



**Fig. 8.** The effect of L-leucine (150 mg. per kilogram) on blood glucose, plasma lactate, and plasma phosphorus.

may not be the result of enhanced uptake of glucose in the limb. On the other hand, the ratio found at 45 minutes in patient C. D. is consistent with an increase in peripheral glucose uptake.

**Effect of leucine on plasma insulin.** The preceding observations, including the insulin-like effect of leucine in these patients on plasma lactate, phosphorus, potassium, the modification of the hyperglycemic response to epinephrine, the likeness between the rate of change in blood glucose concentration obtained with insulin and leucine (Fig. 9), and the failure to detect a direct effect of leucine on erythrocyte metabolism, suggested that leucine, isoleucine, and their keto-acid analogues had an indirect insulin-like action. This notion was confirmed by measuring directly changes in the concentration of plasma insulin following a leucine load in patients C. D. and T. O. by the immunochemical method of Yalow and Berson.[34, 35*] Figs. 10*A* and 10*B* demonstrate the significantly sharp rise in circulating insulin evoked by leucine correlated with the curve of blood glucose in patients C. D. and T. O., respectively. In them the insulin content rose to fourteen times the fasting concentration within 15 to 45 minutes, and subsequently gradually declined. Similar results were obtained after the injection of $\alpha$-ketoisocaproic acid intravenously. It is of interest that the "fasting" level of plasma insulin was not elevated in either patient.

**Effect of two doses of leucine.** To assess the effect of administering a second dose of leucine soon after an initial dose on the plasma concentration of glucose, two doses of L-leucine, 50 mg. per kilogram each, were fed to patients C. D. and T. O. In C. D. the second feeding was administered 100 minutes after the initial one, and, in T. O., 70 minutes following the first dose of L-leucine. The data which are plotted in Figs. 11*A* and 11*B* indicate that a second fall in the concentration of blood glucose was elicited by the repeat load of leucine in both subjects. In patient C. D. there seemed to be a diminished homeostatic response to hypoglycemia following the second leucine feeding. In both subjects indirect evidence of a rise in the concentration of plasma insulin coincident with each dose of leucine was obtained.

## OTHER STUDIES

**Effect of ACTH on blood glucose.** Twenty international units of a gel preparation of adrenocorticotropin was injected intramus-

*We are indebted to Dr. R. Yalow and Dr. S. Berson for the plasma insulin determinations.

cularly on 3 successive days. The fasting concentration of blood glucose following the third injection was 33 mg. per 100 ml.

**Family studies.** The effect of leucine on the blood glucose concentration of the parents and siblings was studied. Two members of the O. kinship, the mother and one sister, showed a moderate fall in the concentration of blood glucose 30 minutes after a leucine feeding (Fig. 12). No effect was observed in the father or other siblings. The response in the propositus T. O. is shown for comparison. A significant change in the blood glucose levels of the mother of C. D. was not obtained.

**Leucine and α-ketoisocaproic acid tolerance tests in other forms of hypoglycemia and in normal subjects.** Three normal subjects and 5 children who at one time exhibited fasting hypoglycemia were tested with leucine (Fig. 13); none showed a significant decrease in the concentration of blood glucose. Two patients with hypoglycemia (G. W. and S. M.) who failed to show a positive leucine test also did not respond to the administration of α-ketoisocaproic acid.

A 42-year-old woman with a metastatic functional islet cell carcinoma of the pancreas who had multiple endocrine adenomatosis was insensitive to leucine; there was no significant drop in blood glucose levels obtained serially at 15 minute intervals for one hour, nor was an increase in circulating insulin detected by immunoassay.

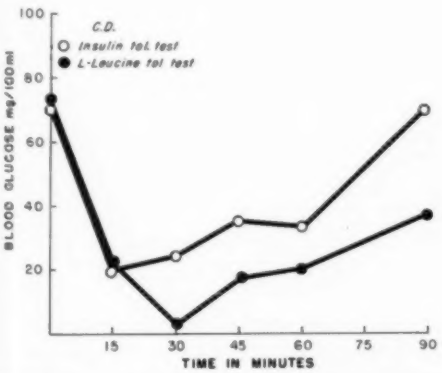



Fig. 9. Comparison of the effect of L-leucine (150-mg. per kilogram by mouth) and of insulin (0.15 U. per kilogram intravenously) on blood glucose.

**Table III.** Oxidation of $C^{14}$ glucose to $CO_2$ by whole blood incubated with and without L-leucine

| Subject | No leucine | | 0.3 mg./ml. L-leucine | |
|---|---|---|---|---|
| | 1-$C^{14}$* | u-$C^{14}$ | 1-$C^{14}$ | u-$C^{14}$ |
| Patient C. D.† | 635 | 168 | 723 | 158 |
| Patient T. O. | 630 | 140 | 650 | 160 |
| Normal | 723 | 178 | 680 | 164 |

*Counts per minute per 2 hr. Two milliliters of whole blood incubated with 0.6 μc of carbon 1 labeled and uniformly labeled $C^{14}$-glucose and 180 mg. per 100 ml. glucose with or without added leucine in a total volume of 3 ml. with Krebs-Ringer-phosphate solution. $CO_2$ counted as Ba $CO_3$ at infinite thickness.

†This patient had a severe deficiency of erythrocyte glucose 6-phosphate dehydrogenase activity.

**Table IV.** Capillary, arterial and venous blood sugar differences in a limb of patient C. D.

| Blood sugar concentration | "Fasting" | 30 minutes after leucine | 45 minutes after leucine |
|---|---|---|---|
| Capillary | 67 | 25 | 29 |
| Venous | 63 | 26 | 24 |
| Capillary minus venous | +4 | −1 | +5 |
| Capillary minus venous/capillary | +0.059 | −0.040 | +0.172 |
| Arterial | | 29 | |
| Venous | | 30 | |
| Arterial minus venous | | −1 | |
| Arterial minus venous/arterial | | −0.034 | |

## DISCUSSION

The results of these studies have characterized further the nature of the defect in glucose homeostasis in the L-leucine-sensitive type of infantile and childhood hypoglycemia. When L-isoleucine or L-leucine was fed, blood glucose was lowered sharply, whereas an effect was not obtained with the D-enantiomorph of leucine. Similarly, the administration of the α-keto acid analogue of L-leucine, α-ketoisocaproic acid, resulted in a marked decrease in the concentration of blood glucose. It seems likely that the keto

acid analogue of L-isoleucine should have a similar effect; however, this compound was not available for study. In contrast to the effect obtained with an impure commercial preparation of isovaleric acid by Cochrane and his associates,[23] in the present study a significant fall in blood glucose content was not found with the use of a highly purified preparation. The results suggest that the hypoglycemia is directly associated either with the keto acid analogues of L-leucine and L-isoleucine, or with these branched chained amino acids, or with both the keto acid and amino acid. Since the transamination step which involves the conversion of the amino acid to its keto acid analogue is reversible[36, 39] present evidence does not permit these distinctions. The failure to induce a fall in blood glucose concentration in response to the administration of isovaleric acid is evidence that the hypoglycemic effect is not related to a metabolite of α-ketoisocaproic acid, the precursor in the metabolic sequence of isovaleric acid. Other degradative products of leucine were not tested, however, and additional studies are needed to substantiate this point. It has been shown previously[23] that tyrosine does not induce hypoglycemia in these patients; one of the metabolites of tyrosine, acetoacetate, is also a product of leucine metabolism.

In the experiments designed to clarify the mechanism of the amino acid– and keto acid–induced hypoglycemia, evidence was obtained which suggests that L-leucine, L-isoleucine, and α-ketoisocaproic acid indirectly exert an insulin-like effect. When a method for determining the insulin content of human plasma became available it was possible to show that the administration of leucine to patients C. D. and T. O. resulted in a striking rise and then a fall in the concentration of circulating insulin which correlated inversely with the changes in blood glucose content.

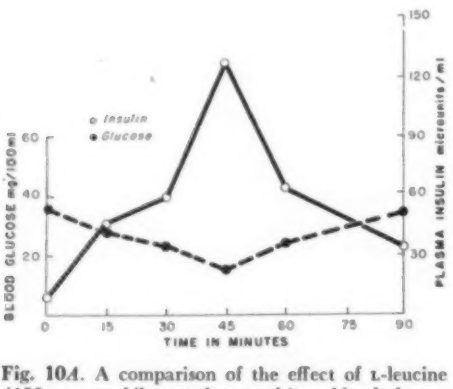



**Fig. 10*A*.** A comparison of the effect of L-leucine (150 mg. per kilogram by mouth) on blood glucose and plasma insulin in patient C. D.

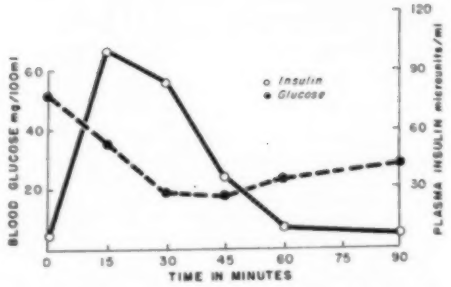



**Fig. 10*B*.** A comparison of the effect of L-leucine (150 mg. per kilogram by mouth) on blood glucose and plasma insulin in patient T. O.

There are at least two possible mechanisms for the hyperinsulinism induced by amino acid or keto acid. These aforementioned substances may cause (a) either directly or indirectly an increase in the rate of production and/or release of insulin by the islets of Langerhans, or (b) a decrease in the rate of destruction of circulating endogenous insulin as a consequence, for example, of the inactivation or inhibition of insulinase,[52-56] an enzyme system which catalyzes the cleavage of the insulin molecule. A third mechanism, tentatively suggested previously among other possibilities by Cochrane[23, 57] that of augmentation of the peripheral action of insulin, does not appear to be of major importance in view of the increased plasma insulin content. Likewise, it seems improbable that the increased concentration of plasma insulin is consequent to inhibition of the insulin-inactivating mechanisms. Both the rate and degree of increase in plasma insulin concentration detected in our subjects following a leucine feeding provide a strong argument against the latter mechanism. Be-

son and his associates[58] found that the half-life of circulating insulin is about 35 minutes. Further, substances which inhibit insulin degradation in vivo have not produced as rapid or marked a fall in blood glucose and, by inference, as sharp a rise in plasma insulin as that observed with leucine and related compounds in the present study.[52, 55]

Hence, it seems probable that these compounds stimulated the secretion of insulin in our patients. Although the possibility that their site of action was extrapancreatic and that the effect on the islets of Langerhans was mediated by way of some insulinotropic or neural mechanism cannot be excluded, we favor the notion of a direct effect on the secretion of insulin by beta cells. A neurogenic mechanism of insulin secretion has been demonstrated, but it appears to be an accessory rather than a fundamental factor since the transplanted or denervated pancreas has been shown to be still capable of regulating insulin secretion. On the other hand, ample data support the contention that increase in the concentration of blood glucose induced by a variety of agents directly stimulates insulin secretion. In addition to hyperglycemia,[59, 60] sulfonylurea derivatives stimulate insulin secretion from the pancreas even in instances in which the blood sugar level is reduced, leading eventually to an increase in islet tissue.[61, 62*] Although the concept that certain sulfonylurea compounds act directly on the beta cells of the islets has been advanced, this mechanism is not yet proved. It should be emphasized that leucine, isoleucine, and α-ketoisocaproic acid in the dosage employed in our studies do not induce hyperinsulinism in the normal subject. Hence, their effect on the insulin-secreting mechanism is peculiar to some individuals with idiopathic hypoglycemia of infancy.

That this action is not limited to this group of patients alone is suggested by an observation which has been brought to our attention recently in an adult[62] and in a child,[63] each with an islet cell adenoma, who had hypoglycemic responses to leucine. In a patient with multiple islet cell tumors and metastases whom we tested with leucine, neither hypoglycemia nor elevation of the plasma insulin level was detected. The data suggest that leucine evokes insulin secretion in some cases of islet cell adenoma, further supporting the notion of a direct betacytotropic action. In addition, 3 patients with maple syrup disease exhibited hypoglycemia,[64] a finding which may result from the strikingly elevated concentration of serum leucine found in this condition.[43, 64]

It would seem significant that an infant with leucine-induced hypoglycemia described by Cochrane and associates[23] showed islet cell hyperplasia when laparotomy was performed

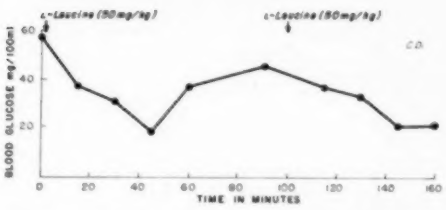



**Fig. 11*A*.** The effect of two feedings of L-leucine on the concentration of blood glucose in patient C. D.

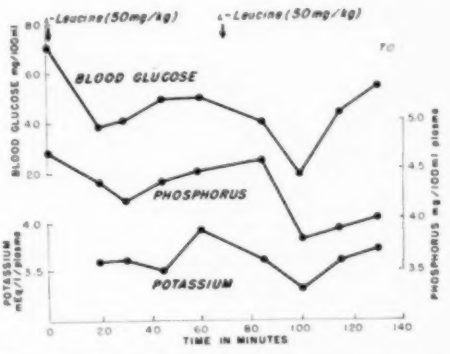



**Fig. 11*B*.** The effect of two feedings of L-leucine on the concentration of blood glucose in patient T. O. The lower figure includes changes in plasma potassium and phosphorus content in T. O.

*Recently we have found that tolbutamide (25 mg. per kilogram intravenously) did not induce a significant fall in blood glucose content in one of our patients. This finding suggests that leucine and tolbutamide may induce the release of insulin from the islet cells by different mechanisms. (See addendum.)

at the age of 14 months, whereas a 2½-month-old infant showed only some "enlargement, vacuolation and loss of granular distinction in the β-cells." These observations seem to indicate that leucine and related compounds may bring about after a sufficient interval secondary hyperplasia and increased growth of the islet tissue in this group of patients. The latter may be considered a morphologic reflection of an abnormality in the mechanisms regulating insulin secretion.

The plasma concentration of insulin in the "fasting" state was within the limit of normal values in our 2 affected patients (C. D. and T. O.). Further, plasma insulin activity, as determined by the rat-diaphragm method, was increased in only 1 of 3 patients studied by others.[23] Nonetheless, fasting hypoglycemia is an important characteristic of this disorder. The apparent disparity between the concentrations of blood glucose and of plasma insulin in the fasting state may be related to the effect of small amounts of endogenous insulin on hepatic glucose output[50, 51] and to binding of insulin by the liver. On the other hand, it is quite likely that the secretion of insulin in leucine-induced hypoglycemia is intermittent. Since the insulin level in the peripheral circulation falls rapidly because of dilution and degradation, the concentration of insulin in a random sample of plasma, even though obtained at a time when the blood glucose content is low, may not be elevated.

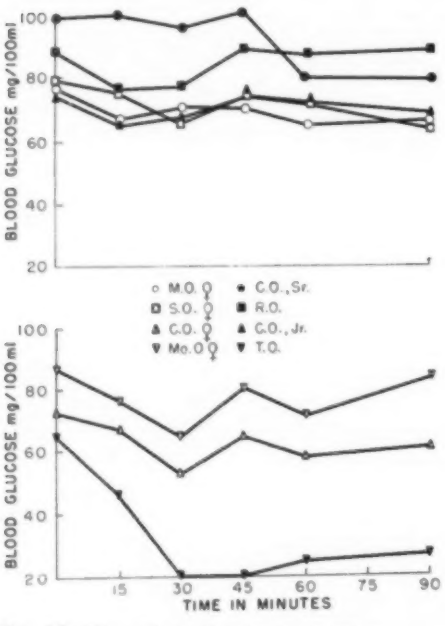



**Fig. 12.** The effect of L-leucine (150 mg. per kilogram by mouth) on blood glucose in the O. kinship.

There is evidence that the leucine-sensitive type of "idiopathic" hypoglycemia is a familial disorder.[23] The mother and the sister of T. O. each showed a moderate fall in the concentration of blood glucose when leucine was fed and both had vague symptoms suggestive of mild hypoglycemia. The father and 4 other siblings did not show a blood glucose response when tested, nor did the mother of C. D. The familial data while highly suggestive of a heritable disorder are insufficient to indicate the pattern of inheritance.

The clinical aspects of leucine-induced hypoglycemia as manifested in our 2 patients exhibited a distinctive feature which was brought out by a detailed dietary history. Although these patients frequently have fasting hypoglycemia, symptoms were not commonly observed before breakfast. The lack of symptoms may be related to a slow rate of glucose fall during fasting periods. Nevertheless, there was good correlation between what proved to be hypoglycemic reactions and the ingestion of meals containing proteins with a high leucine content such as milk, eggs, fish, or meat. The pattern of recurrent seizures, drowsiness, weakness, or stupor shortly after a meal should arouse suspicion of this form of hypoglycemia.

It should be emphasized that not all cases of idiopathic hypoglycemia, as defined by McQuarrie,[5] are of the leucine-sensitive type. It is now apparent that this syndrome may result from a variety of disorders, the etiologies of which are still obscure. Now that a method is available for the direct estimation of insulin in human plasma it should be possible to distinguish those instances associated with an absolute increase in circulating insulin from those in which there is

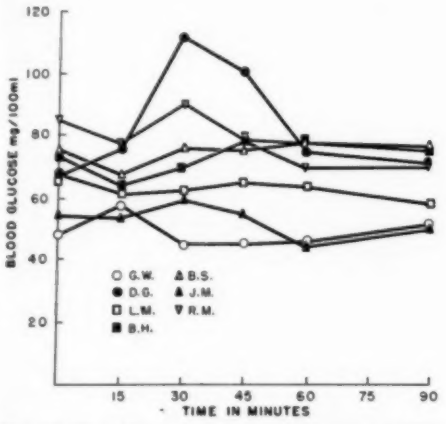



**Fig. 13.** The effect of L-leucine (150 mg. per kilogram by mouth) on the blood sugar of children with fasting hypoglycemia and in normal subjects.

relative hyperinsulinism and to investigate other substances which in this syndrome may affect the secretion of insulin. In turn this should lead to a clearer rationale for therapy.

There are several approaches which merit consideration in the treatment of the leucine-sensitive type of idiopathic hypoglycemia. In our experience and that of Cochrane and associates,[23] feeding a glucose or sucrose supplement between 15 and 45 minutes after a meal, at bedtime, and early in the morning has resulted in considerable symptomatic improvement. No additional treatment was required in patients C. D. and T. O. In some instances it may be necessary to reduce the leucine and isoleucine content of the diet. However, both of these substances are essential amino acids and their minimal dietary requirements* must be met.[69, 70] In resistant cases a repository preparation of glucagon or epinephrine may be of value. Ephedrine administered by mouth to one of our patients did not modify the hypoglycemic effect of leucine.

Not all cases of idiopathic hypoglycemia show a good response to treatment with adrenocorticotropin or corticosteroids. This has been especially true of the leucine-sensitive group,[16, 23] perhaps in part because of the intermittent secretion of insulin. The effect of large doses of a repository preparation of adrenocorticotropin on the fasting blood glucose concentration was disappointing in one of our patients. On the other hand, this form of treatment has proved of value in some patients with islet cell adenoma, and should be tried in severe cases.[23, 71] Parenthetically, it should be mentioned that neither the response to adrenocorticotropin nor the response to leucine can exclude an islet cell adenoma in patients with hypoglycemia. Finally, in the management of the leucine-sensitive group subtotal pancreatectomy should be considered when other measures fail. This procedure did not result in improvement in one of Cochrane's patients[23]; however, it seems likely that removal of a sufficient amount of islet tissue should result in an increase in the fasting blood glucose content and in a modification of the hypoglycemic response to leucine.

*Rose and his associates,[69] using the nitrogen balance technique, have suggested that for the adult a "safe" intake (which they arbitrarily define as twice the minimal requirement) of leucine is 2.2 Gm. per day and of isoleucine 1.4 Gm. per day. On the other hand, Snyderman and Holt[70] have obtained data which indicate that the requirement of these two amino acids for the infant is about five times as great as that for the adult.

## SUMMARY

1. Studies on 2 patients with leucine-induced "idiopathic" hypoglycemia showed that L-isoleucine also caused a striking fall in the concentration of blood glucose. D-leucine, L-valine, L-alanine, DL-threonine, and L-glycine did not have a significant effect.

2. The α-keto acid analogue of leucine, α-ketoisocaproic acid, induced hypoglycemia when injected intravenously but not its metabolite isovaleric acid. Its action was less effective orally. Pyruvic acid and α-ketoisovaleric acid failed to elicit a similar response.

3. No evidence of a defect in amino acid or α-keto acid metabolism was found, nor of a direct effect of leucine on glycolysis in whole blood.

4. It was shown that leucine caused increased peripheral utilization of glucose, suggesting an insulin-like action. Direct determinations of plasma insulin indicated a sharp rise in circulating insulin following the administration of leucine.

5. It is concluded that L-leucine, L-isoleu-

cine, and their keto acid analogues induce an increase in the concentration of plasma insulin irrespective of the blood glucose level, an effect which may be attributed to their direct action on the mechanism of insulin secretion.

## ADDENDUM

We have had the opportunity of studying a third patient, E. A., a 13-month-old boy, with leucine-sensitive hypoglycemia in whom the diagnosis was suggested prior to laboratory investigation by the "typical" history obtained. A significant fall in the concentration of blood glucose was not obtained following the intravenous administration of 25 mg. per kilogram of tolbutamide which suggests that leucine and tolbutamide may affect the release of insulin from the beta cells through different mechanisms. The patient showed a fall in blood glucose content after the administration of L-leucine, L-isoleucine, and $\alpha$-ketoisocaproic acid comparable to that obtained in C. D. and T. O.

---

We are greatly indebted to Dr. R. S. Yalow and Dr. S. A. Berson for the determinations of plasma insulin, and to Dr. S. Moore and Dr. M. P. Brigham for the measurement of plasma amino acids.

---

A paper in two parts on "Leucine-Induced Hypoglycemia" by C. Charlton Mabry, M.D., Angelo M. DiGeorge, M.D., and Victor H. Auerbach, Ph.D., will appear in the October issue. This paper and the one appearing here were submitted for publication at essentially the same time. *Ed.*

## REFERENCES

1. Heberden, P., and Friedlander, F.: Hypoglycaemic Coma, Arch. Dis. Childhood **30:** 372, 1955.
2. Garland, H.: Endogenous Hypoglycaemia, Proc. Roy. Soc. Med. **51:** 979, 1958.
3. Combined Staff Clinics: Hypoglycemia, Am. J. Med. **1:** 412, 1946.
4. Lawrence, R. D., Meyer, A., and Nevin, S.: The Pathological Changes in the Brain in Fatal Hypoglycaemia, Quart. J. Med. **11:** 181, 1942.
5. McQuarrie, I.: Idiopathic Spontaneously Occurring Hypoglycemia in Infants. Clinical Significance of Problem and Treatment, A. M. A. Am. J. Dis. Child. **87:** 399, 1954.
6. Darrow, D. C.: Mental Deterioration Associated With Convulsions and Hypoglycemia, Am. J. Dis. Child. **51:** 575, 1936.
7. Gardner, L. I., and Reyersbach, G. C.: Brain Damage in a Juvenile Diabetic Patient Associated With Insulin Hypoglycemia, Pediatrics **7:** 210, 1951.
8. Tom, M. I., and Richardson, J. C.: Hypoglycaemia From Islet Cell Tumor of Pancreas With Amyotrophy and Cerebrospinal Nerve Cell Changes; A Case Report, J. Neuropath. & Exper. Neurol. **10:** 57, 1951.
9. Conn, J. W., and Seltzer, H. S.: Spontaneous Hypoglycemia, Am. J. Med. **19:** 460, 1955.
10. Porter, M. R., and Frantz, V. K.: Tumors Associated With Hypoglycemia—Pancreatic and Extrapancreatic, Am. J. Med. **21:** 944, 1956.
11. Frantz, V. K.: Tumors of the Pancreas, in Atlas of Tumor Pathology, Washington, 1959, Armed Forces Institute of Pathology.
12. Skillern, P. G., and Rynearson, E. H.: Medical Aspects of Hypoglycemia, J. Clin. Endocrinol. **13:** 587, 1953.
13. Kinsbourne, M., and Woolf, L. I.: Idiopathic Infantile Hypoglycaemia, Arch. Dis. Childhood **34:** 166, 1959.
14. Broberger, O., Jungner, I., and Zetterstrom, R.: Studies in Spontaneous Hypoglycemia in Childhood. Failure to Increase the Epinephrine Secretion in Insulin-Induced Hypoglycemia, J. Pediat. **55:** 713, 1959.
15. Douglas, D. M.: Spontaneous Hyperinsulinism Due to Benign Hyperplasia of Islet Cells, Arch. Dis. Childhood **34:** 171, 1959.
16. Royer, P., Lestradet, H., and Cachin, O.: L'Hypoglycémie fonctionnelle récurrente corticosensible de l'enfant, Arch. franç. pédiat. **16:** 69, 1959.
17. Froesch, E. R., Prader, A., Wolf, H. P., and Labhart, A.: Die hereditare Fructoseintoleranzz Helvet. paediat. acta **14:** 99, 1959.
18. Stuart, K. L., Jelliffe, D. B., and Hill, K. R.: Acute Toxic Hypoglycemia Occurring in Vomiting Sickness of Jamaica (Clinical Aspects), J. Trop. Pediat. **1:** 69, 1955.
19. Howard, J. M., Moss, N. H., and Rhoads, J. E.: Hyperinsulinism and Islet Cell Tumors of the Pancreas With 398 Recorded Tumors, Internat. Abstr. Surg. in Surg., Gynec. & Obst. **90:** 417, 1950.
20. Roxburgh, R. C.: Islet Cell Adenoma of the Pancreas (in a Child Age 7), Lancet **1:** 1057, 1954.
21. Klein, H., and Klein, S. P.: Spontaneous Hypoglycemia Associated With Massive Hepatoma, A. M. A. Arch. Int. Med. **103** 273, 1959.
22. Miller, D. R., Bolinger, R. E., Janican, D. Crockett, J. E., and Friesen, S. R.: Hypoglycemia Due to Nonpancreatic Mesodermal Tumors: Report of Two Cases, Ann. Surg **150:** 684, 1959.
23. Cochrane, W. A., Payne, W. W., Simpkiss M. J., and Woolf, L. I.: Familial Hypoglycemia Precipitated by Amino Acids, J. Clin Invest. **35:** 411, 1956.

24. Saifer, A., and Gerstenfeld, S.: The Photometric Microdetermination of Blood Glucose With Glucose Oxidase, J. Lab. & Clin. Med. **51:** 448, 1958.

24a. Von Euler, U. S., and Lishajako, F.*: The Estimation of Catechol Amines in Urine, Acta physiol. scandinav. **45:** 122, 1959.

25. Benedict, S. R.: The Analysis of Whole Blood. II. The Determination of Sugar and of Saccharoids (Non-fermentable Copper-Reducing Substances), J. Biol. Chem. **92:** 141, 1931.

26. Barker, S. B., and Summerson, W. H.: The Colorimetric Determination of Lactic Acid in Biological Material, J. Biol. Chem. **138:** 535, 1941.

27. Fiske, C. H., and SubbaRow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66:** 375, 1925.

28. Moore, S., and Stein, W. H.: Procedures for the Chromatographic Determination of Amino Acids on Four Per Cent Cross-Linked Sulfonated Polystyrene Resins, J. Biol. Chem. **211:** 893, 1954.

29. Dent, C. E.: A Study of the Behaviour of Some Sixty Amino Acids and Other Ninhydrin-Reacting Substances on Phenol-"Collidine" Filter Paper Chromatograms, With Notes as to the Occurrence of Some of Them in Biological Fluids, Biochem. J. **43:** 169, 1948.

30. Van Slyke, D. D., MacFayden, D. A., and Hamilton, P. B.: The Gasometric Determination of Amino Acids in Urine by the Ninhydrin-Carbon Dioxide Method, J. Biol. Chem. **150:** 251, 1943.

31. Menkes, J. H.: The Pattern of Urinary Alpha Keto Acids in Various Neurological Diseases, A. M. A. J. Dis. Child. (in press).

32. Wilson, H.: Chromogenic Values of Various Ketosteroids in a Micro Modification of the Zimmerman Reaction: Comparison With the Macro Procedure, Arch. Biochem. **52:** 217, 1954.

33. Birke, G., Diczfalusy, E., and Plantin, L.: Assessment of the Functional Capacity of the Adrenal Cortex. I. Establishment of Normal Values, J. Clin. Endocrinol. **18:** 736, 1958.

34. Yalow, R. S., and Berson, S. A.: Assay of Plasma Insulin in Human Subjects by Immunological Methods, Nature **184:** 1648, 1959.

35. Berson, S. A., and Yalow, R. S.: Immunoassay of Insulin, in Antoniades, H., editor: Hormones in Human Plasma, Boston, Little, Brown & Company (in press).

36. Coon, M. J., Robinson, W. G., and Bachhawat, B. K.: In McElroy, W. D., and Glass, B., editors: Amino Acid Metabolism, Baltimore, 1955, Johns Hopkins Press, p. 431.

37. Robinson, W. G., Bachhawat, B. K., and Coon, M. J.: Tiglyl Coenzyme A and α-Methylacetoacetyl Coenzyme A, Intermediates in the Enzymatic Degradation of Isoleucine, J. Biol. Chem. **218:** 391, 1956.

38. Kinnory, D. S., Takeda, Y., and Greenberg, D. M.: Isotopes Studies on the Metabolism of Valine, J. Biol. Chem. **212:** 385, 1955.

39. Radhakrishnan, A. N., and Meister, A.: Amino Acid Synthesis by Reversal of the Amino Acid Oxidase Reaction, J. Biol. Chem. **233:** 444, 1958.

40. Mabry, C. C., DiGeorge, A. M., Auerbach, V. H., and Reardon, H.: Additional Observations Concerning the Defect in an Infant With Hypoglycemia Precipitated by Leucine (Abstract), Proc. Soc. Pediat. Res., 1959, p. 52.

41. Snyderman, S. E., Cusworth, D. C., Roitman, E., and Holt, L. E., Jr.: Amino Acid Interrelationships: The Effect of Variations in Leucine Intake (Abstract) Fed. Proc. **18:** 546, 1959; and Personal communication.

42. Luck, J. M., Morrison, G., and Wilbur, L. F.: The Effect of Insulin on the Amino Acid Content of Blood, J. Biol. Chem. **77:** 151, 1928.

43. Dancis, J., Levitz, M., and Westall, R. G.: Maple Syrup Urine Disease: Branched Chain Keto-Aciduria, Pediatrics **25:** 72, 1960.

44. Marks, P. A.: Enzyme Methods in Clinical Research: Enzymes of the Pentose Phosphate Pathway, in Methods in Medical Research, Chicago, 1960, The Year Book Publishers, Inc., vol. 9 (in press).

45. Soskin, S., Levine, R., and Hechter, O.: The Relation Between the Phosphate Changes in Blood and Muscle, Following Dextrose, Insulin and Epinephrin Administration, Am. J. Physiol. **134:** 40, 1941.

46. Kaplan, N. O., and Greenberg, D. M.: Radioactive Phosphate as an Indicator of the Relationship Between the Phosphate Changes of Blood, Muscle and Liver, Following the Administration of Insulin, Am. J. Physiol. **140:** 598, 1944.

47. Nichols, N.: The Effect of Glycogen Deposition on Liver Phosphorus, J. Clin. Invest. **34:** 1710, 1955.

48. Stadie, W. C.: Current Concepts of the Action of Insulin, Physiol. Rev. **34:** 52, 1954.

49. Bell, D. M., and Burns, T.: The Effect on Femoral A-V Glucose Difference of Insulin Injection Into an Antecubital Vein and Into a Femoral Artery, J. Clin. Invest. **31:** 717, 1952.

50. Madison, L. L., and Unger, R. H.: The Physiologic Significance of the Secretion of Endogenous Insulin Into the Portal Circulation. I. Comparison of the Effects of Glucagon-Free Insulin Administered Via the Portal Vein and Via a Pheripheral Vein on the Magnitude of Hypoglycemia and Peripheral Glucose Utilization, J. Clin. Invest. **37:** 631, 1958.

51. Madison, L. L., Combes, B., Strickland, W., Unger, R., and Adams, R.: Evidence for a Direct Effect of Insulin on Hepatic Glucose Output, Metabolism **8:** 469, 1959.

*The method used is more closely related to that of Von Euler and Lishajako than to the older procedure of Von Euler and Floding.

52. Mirsky, I. A.: Insulinase, Insulinase-Inhibitors, and Diabetes Mellitus, Recent Prog. in Hormone Research **13:** 429, 1957.
53. Mirsky I. A., Perisutti, G., and Diengott, D.: The Hypoglycemic and Insulinase Inhibitory Action of L-Tryptophan, Endocrinology **59:** 369, 1956.
54. Mirsky, I. A., and Perisutti, G.: The Relative Specificity of the Insulinase Activity of Rat Liver Extracts, J. Biol. Chem. **228:** 77, 1957.
55. Williams, R. H., Martin, F. B., Henley, E. D., and Swanson, H. E.: Inhibitors of Insulin Degradation, Metabolism **8:** 99, 1959.
56. Tomizawa, H. H., and Halsey, Y. D.: Isolation of an Insulin-Degrading Enzyme From Beef Liver, J. Biol. Chem. **234:** 307, 1959.
57. Cochrane, W. A., and Moya, F.: The Relationship of Amino Acids to Idiopathic Hypoglycemia (Abstract), Proc. Soc. Pediat. Res., 1959, p. 18.
58. Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K.: Insulin-$I^{131}$ Metabolism in Human Subjects: Demonstration of Insulin Binding Globulin in the Circulation of Insulin Treated Subjects, J. Clin. Invest. **35:** 170, 1956.
59. Anderson, E., and Long, J. A.: The Effect of Hyperglycemia on Insulin Secretion as Determined With the Isolated Rat Pancreas in a Perfusion Apparatus, Endocrinology **40:** 92, 1947.
60. Brown, E. M., Jr., Dohan, F. C., Freedman, L. R., De Moor, P., and Lukens, F. D. W.: The Effects of Prolonged Infusion of the Dog's Pancreas With Glucose, Endocrinology **50:** 644, 1952.
61. Loubatières, A.: The Mechanism of Action of Hypoglycemic Sulfonamides. Concept Based on Investigation in Animals and Man, Diabetes **6:** 408, 1957.
62. Yalow, R. S., and Berson, S. A.: Personal communication.
63. Crigler, J.: Personal communication.
64. Menkes, J.: a. Personal communication. b. Maple Syrup Disease. Isolation and Identification of Organic Acids in the Urine, Pediatrics **23:** 348, 1959.
65. Levine, R., and Fritz, I. B.: The Relation of Insulin to Liver Metabolism, Diabetes **5:** 209, 1956.
66. DeBodo, R. C., Steele, R., Altszuler, N., Dunn, A., Armstrong, D. T., and Bishop, J. S.: Further Studies on the Mechanism of Action of Insulin, Metabolism **8:** 520, 1959.
67. Cahill, G. F., Jr., Ashmore, J., Renold, A. E., and Hastings, A. B.: Blood Glucose and the Liver, Am. J. Med. **26:** 264, 1959.
68. De Duve, C.: The Hepatic Action of Insulin, Ciba Foundation Colloquia on Endocrinol. **9:** 203, 1956.
69. Rose, W. C., Wixom, R. L., Lockhart, H. B., and Lambert, G. F.: The Amino Acid Requirements of Man. XV. The Valine Requirement; Summary and Final Observations, J. Biol. Chem. **217:** 987, 1955.
70. Snyderman, S. E., and Holt, L. E., Jr.: Personal communication.
71. McQuarrie, I., Ziegler, M. R., Wright, W. S., Bauer, E. G., and Ulstrom, R. A.: Further Studies on the Effects of ACTH on Spontaneous Hypoglycemias, in Mote, J. R., editor: Proc. Second Clin. ACTH Conf., Philadelphia, 1951, The Blakiston Company, p. 69.

# Human growth hormone

## II. Further study of its effect on growth in dwarfism

Thomas H. Shepard, II, M.D.,* Sorrel Waxman, M.D.,

Nan Bernstein, M.S., and Pierre Ferrier, M.D.

SEATTLE, WASH.

ALTHOUGH human growth hormone has been shown by a number of workers[1-7] to be metabolically effective in hypopituitary dwarfism, there is still relatively little knowledge about resultant linear growth and maturation of bone.[2, 3, 4] The optimal dosage schedule and mode of administration has not been decided finally, and its long-term effects on other organ systems (nervous, muscular, renal, reproductive, and hemopoietic) are not known. Little has been reported on the effects of human growth hormone in conditions other than hypopituitarism.

The purpose of this paper is to give a further report of the effect of human growth hormone in a "hypopituitary" child. Detailed metabolic balance and hormonal studies were reported in a previous paper.[7] In addition, results of studies carried out on a constitutional or primordial dwarf are given.

### METHODS AND MATERIALS

The linear measurements and the chemical determinations were made as previously reported.[7, 8] The growth hormone was extracted by Dr. Maurice Raben from human pituitaries collected by the authors at autopsy.

Patient 1 was a 3 10/12-year-old Japanese boy with a typical course and laboratory findings of hypopituitarism. The details have been previously reported.[7] This "hypopituitary" dwarf received 5 mg. of human growth hormone (Raben[12]) daily during an initial 10-day balance study and was then given 2 mg. every other day for a period of 2 months, at the end of which he received 5 mg. daily for 11 days. After a 1-month interval without treatment, the administration of 3 mg. every other day was continued for 7 months. Height measurements are shown in Figs. 2 and 3 and Table I.

Patient 2 was a 7-year-old Japanese boy who was premature at birth. Weight and linear growth had progressed along a line that is parallel to but 6 cm. below the third percentile for American males[10] (Fig. 6). In addition, a shortened right leg, severe dental caries, and small curved fifth fingers were present.

The mother's pregnancy was normal but the birth occurred 3 weeks before term. The weight at birth of 2,100 grams necessitated a 21-day stay in the nursery, during which he progressed satisfactorily. It was not possible to accurately assess the state of his ma-

*From the Department of Pediatrics, University of Washington, Seattle, Wash.*

*Supported by a grant from the Boeing Good Neighbor Fund and by Playtex Park Research Institute, Grant No. 74TR, and by a training grant from the National Institute of Arthritis and Metabolic Disease (2A-5190).*

**Address, Department of Pediatrics, University of Washington School of Medicine, Seattle 5, Wash.*

**Fig. 1A.** Patient 1 before therapy.

turity by history. His subsequent nutritional and developmental history were within normal limits, and his current scholastic work in the second grade has been excellent. The parents were of normal heights (152 cm. and 160 cm.) and there were no adult relatives under 5 feet in height.

On physical examination when he was $5\frac{3}{12}$ years of age he was small, well proportioned, alert, and active. His physical measurements are shown in Table II. This stature was definitely reduced as compared with Japanese standards.[11] The dental enamel appeared thin and marked caries were present; this had led to the removal of 3 deciduous teeth. Although no simian palmar lines were present, both fifth fingers were shortened and curved. The lower extremities were of different length; the right measured 2.5 cm. less than the left, and the circumference of the right thigh and calf were reduced. The remainder of the physical examination was not remarkable.

X-rays revealed the following pertinent calcified epiphyseal centers: triquetrum, greater tuberosity of the shoulder, great trochanter, and third cuneiform. The epiphyses of the proximal fibula, patella, and navicular were not seen. The bony maturation was judged to be in the 3 to 4½-year range. Skull x-rays were normal as were routine urine and hematologic studies. Further chemical data are presented in Table IV. Two follicle-stimulating hormone assays carried out on urine were negative at 3 uterine M.U.

This child with genetic-like dwarfism was given 5 mg. of human growth hormone daily during a 10-day period and then 7 mg. twice weekly for 1 week. During a 5-day period before therapy and during the first 10-day period during therapy all urine was collected, measured, and analyzed for nitrogen (Table III). An attempt was made to keep his diet relatively constant during this time and his daily nitrogen intake was

**Fig. 1B.** Patient 1 after 10 months of treatment. Note the increase in height and decrease in adipose tissue of abdomen and cheeks.

estimated. Blood chemical values are shown in Table IV and the changes in height are illustrated in Figs. 6 and 7.

## RESULTS AND DISCUSSION

**"Hypopituitary" dwarf.** The immediate results of human growth hormone therapy in hypopituitary subjects is a prompt positive balance for nitrogen, potassium, sodium, calcium, and phosphorus.[1-7] This was observed in Patient 1 and reported in detail elsewhere.[7] Subsequent blood chemical changes have not been dramatic. The serum phosphorous and alkaline phosphatase have not been elevated.

The increase in height during the first 10 months of treatment was 9.3 cm. which is approximately 4.3 cm. more than the expected increase for an average child of his age during the same period.[10] This rate is over 3 times that observed in the year before therapy. Raben[12] has reported a yearly increase in height of 8.2 cm. in a 17-year-old hypopituitary boy treated with 6 mg. of human growth hormone per week, and Hutchings and colleagues[2] have treated an 11-year-old hypopituitary girl with varying doses of growth hormone, purified by C.H.-Li and observed a 9 cm. increment during 9 months of therapy.

The schedule of doses used during the major portion of this study was 3 mg. every other day given intramuscularly. Results of different schedules of therapy or with smaller amounts are yet to be investigated.

In Fig. 2 it can be seen that a rather prompt increase in height was observed shortly after each course of therapy in which 5 mg. was given daily. The significance of this will be studied by repeating it. The small amount of available growth hormone has prevented administration of 5 mg. for a longer time. The shrinkage which is recorded after each spurt in growth is also of doubtful significance but could be due to reduction in the number of cartilage cells in each epiphyseal plate. The significance and effect of the mild attack of varicella cannot be interpreted. It was clearly demonstrated to the authors that

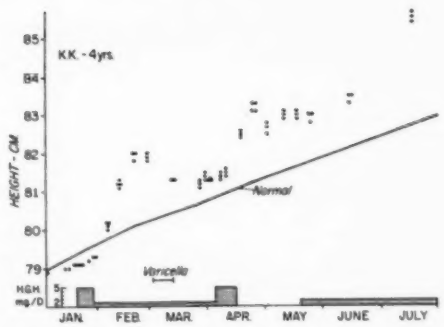



**Fig. 2.** Details of growth measurements and dosage of growth hormone for Patient 1. Each group of dots represents separate measurements recorded on the same day. The line marked normal represents the average rate of increment, not the mean for his age.

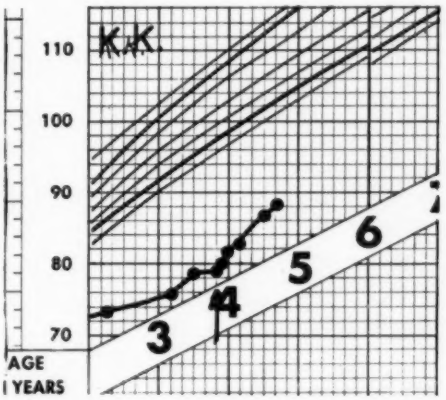



**Fig. 3.** Changes in height of Patient 1 as compared with percentiles of height increments for normal children.[9]

the measurement of height varied during the day, the height in late afternoon being approximately 1 cm. less than that of the early morning.

In spite of rapid growth no "growing pains" were reported and no local or general reactions to the injections were detected.

Changes in body proportions (Table I) were not measurable. The measurement of both the lower segment and span are subject to fairly large errors and the lack of significant change may be due to defects in measurements and the short period of

**Table I.** Measurements of Patient 1

| *Age (years)* | *Height (cm.)* | *Weight (kilograms)* | *Lower segment (cm.)* | *Span (cm.)* | *Head circumference (cm.)* | *Chest circumference (cm.)* | *Abd. circumference (cm.)* | *Lower segment × 100 / height (%)* | *Span × 100 / height (%)* |
|---|---|---|---|---|---|---|---|---|---|
| 2 3/12 | 73.6 | | | | 47 | 47 | 47 | | |
| 3 3/12 | 75.9 | | | | | | | | |
| 3 5/12 | 78.7 | 10.0 | | | | | | | |
| 3 10/12 | 78.9 | 11.5 | 34.2 | 75.0 | | | | 43.3 | 94.9 |
| 3 11/12 | 80.2 | 10.9 | 36.0 | 76.2 | 48.6 | 48 | 47 | 44.9 | 95.0 |
| 4 | 81.9 | 11.6 | 34.9 | 78.4 | 48.5 | 49 | 51 | 42.9 | 95.7 |
| 4 3/12 | 82.6 | 11.2 | 36.0 | 80.3 | 48.5 | 47 | 45 | 43.5 | 97.2 |
| 4 6/12 | 86.9 | 12.0 | 38.8 | 82.0 | 49.2 | 49 | 51.5 | 44.6 | 94.4 |
| 4 7/12 | 87.8 | 13.4 | 39.3 | 83.1 | 49.5 | 48.5 | 45.5 | 44.8 | 94.6 |
| 4 8/12 | 88.2 | 12.5 | 38.8 | 83.3 | 49.4 | 48 | 46 | 44.0 | 94.4 |

**Table II.** Physical measurements of Patient 2

| | | | |
|---|---|---|---|
| Age | 5 3/12 | 7 8/12 | 7 7/12 |
| Height | 96.5 cm. | 107.3 cm. | 108.3 cm. |
| Weight | 12.7 kilograms | 15.7 kilograms | 16.1 kilograms |
| | 47.2 cm. | 52.8 cm. | 52.8 cm. |
| $\frac{\text{Lower segment}}{\text{height}} \times 100$ | 48.9% | 49.2% | 48.7% |
| Span | 90.0 cm. | 100.0 cm. | 100.0 cm. |
| Circumference of head | 50.0 cm. | 51.0 cm. | 51.5 cm. |
| Circumference of chest | 47.0 cm. | 50.0 cm. | 50.5 cm. |
| Circumference of abdomen | 45.0 cm. | 46.0 cm. | 44.0 cm. |

**Table III.** Balance data of Patient 2

| *Day* | *Volume of urine (ml.)* | *Urininary nitrogen (Gm./day)* | *Nitrogen in stools (estimated Gm.)* | *Dietary nitrogen (estimated Gm.)* | *Rx* | *Balance (Gm.)* | *Average* | *Weight (kilograms)* | *Height (cm.)* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 402 | 6.97 | .8 | | None | | | 15.95 | 108.5 |
| 2 | 375 | 6.50 | .8 | 8.57 | None | +1.27 | | 16.0 | 108.4 |
| 3 | 374 | 8.19 | .8 | 6.73 | None | −2.16 | | | |
| 4 | 520 | 12.33 | .8 | 8.98 | None | −4.15 | | 16.1 | 108.6 |
| 5 | 360.8 | 10.47 | .8 | 9.48 | None | −1.79 | −1.71 | | |
| 6 | 391.3 | 11.15 | .8 | 8.61 | 5 mg. HGH | −3.34 | | 15.9 | 108.3 |
| 7 | 356.1 | 7.01 | .8 | 8.03 | 5 mg. HGH | + .22 | | 16.15 | 108.5 |
| 8 | 370.8 | 10.14 | .8 | 6.23 | 5 mg. HGH | −4.71 | | 16.65 | 108.4 |
| 9 | 556 | 9.45 | .8 | 8.26 | 5 mg. HGH | −1.99 | | 16.05 | 108.5 |
| 10 | 462.5 | 15.20 | .8 | 8.70 | 5 mg. HGH | −7.1 | | 16.2 | 108.4 |
| 11 | 405.0 | 6.50 | .8 | 7.20 | 5 mg. HGH | + .1 | | 16.2 | 108.4 |
| 12 | 490.0 | 7.67 | .8 | 7.38 | 5 mg. HGH | −1.09 | | | |
| 13 | 514.3 | 6.33 | .8 | 9.12 | 5 mg. HGH | +3.08 | | 16.3 | 108.4 |
| 14 | 446.5 | 4.44 | .8 | 9.79 | 5 mg. HGH | +4.55 | | 16.2 | 108.2 |
| 15 | 377.5 | 5.24 | .8 | 8.48 | 5 mg. HGH | +2.44 | − .77 | 16.05 | 108.3 |

observation. The increase in head circumference is probably real; no changes were noted in the patient's intellect or personality. The testes and penis remained unchanged. The parents, however, did observe an increase in the number of erections, an observation reported by others in adults.

Maturation of bone during a 9-month period is most difficult to assess. One small new metatarsal epiphysis was seen (Fig. 5)

after 9 months of therapy and there were slight increases in the sizes of the preexisting epiphyses. A point of interest is the probable increase in width of the area of provisional calcification at the ends of the long bones. This correlates with the histologic increase in this area which has been observed in animals treated with growth hormone.[9] Simpson, Asling, and Evans[9] in their extensive investigations of rats upon which hypophysectomies had been performed have reported linear growth without bone maturation in animals treated with growth hormone alone. Their observations in animals seem comparable with the changes observed in Patient 1.

The increase in weight (2.5 kilograms) during 10 months of therapy was not associated with any increase in appetite as reported by the parents. As can be seen in Fig. 1 the accumulation of adipose tissue in the cheeks and abdomen became reduced and brings to mind the fat-mobilizing effect of growth hormone which has recently been reviewed by Raben.[13] It is of interest that the nonesterified fatty acids of the serum have become increased in two other "hypopituitary" children under treatment, but not in Patient 2 nor in premature infants[16] in whom the growth hormone was ineffective.

Fig. 4. Epiphyseal centers of hand before therapy ($3\frac{10}{12}$ years) in Patient 1.

Fig. 5. Epiphyseal centers of Patient 1 after 9 months' therapy. The epiphyses, which are approximately the same size as in Fig. 4, now appear to be more separated from the ends of the long bones.

**Constitutional (primordial) dwarfism.**[14] Constitutional or primordial dwarfism may be defined by the following criteria: (1) premature birth weight associated with full-term delivery; (2) evidence at birth of physiologic and biochemical maturity (i.e., breast hypertrophy, some scaliness of skin, and ability to regain birth weight in a short time); (3) linear growth rate which is parallel to, but significantly less than, 3

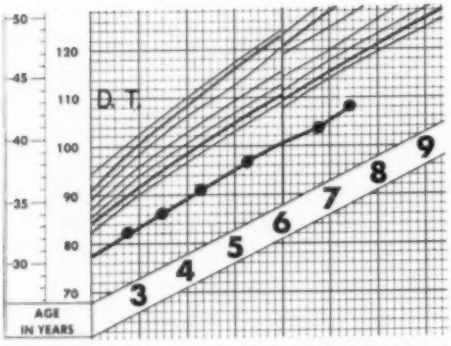



**Fig. 6.** Steady increment in height of Patient 2 as compared with percentiles of height increments for normal children.[9]

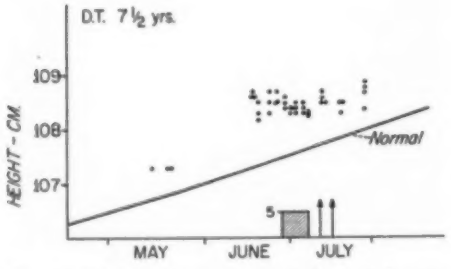



**Fig. 7.** Height measurements of Patient 2 compared with mean increment for age. Each day's measurements are recorded in triplicate and account for the small clusters.

standard deviations from the mean; (4) normal bone maturation and normal time of onset of puberty; and (5) absence of other cause for dwarfing.

Although Patient 2 does not exhibit all of the above criteria, he is best classified in this category. The rampant dental caries has been frequently observed by the authors in other children with this condition and Silver and others[15] have drawn attention to the hemihypertrophy associated with this syndrome. Although no elevation of gonadotropin could be found, it is perhaps pertinent to report that two other constitutional or primordial dwarfs observed by the authors have entered puberty at the age of 6 to 7 years.

The balance study (Table III) leaves much to be desired because a constant measured diet could not be maintained and because stools were not analyzed. It can be said, however, that with a dosage of 5 mg. no dramatic changes in urinary nitrogen were seen. The trend toward a positive balance during the last 3 days might be associated with the increased intake that occurred at that time or might be due to delayed action of growth hormone. The chemical changes (Table IV) in the blood were unremarkable except for the absence of any increase in the fasting nonesterified fatty acids.[13] This child, however, had relatively small amounts of adipose stores. The lack of change in this boy contrasts strongly with the positive findings in hypopituitary children who are treated with growth hormone. The reason for this failure in response could be explained by the inability of exogenous growth hormone to augment a

**Table IV.** Blood chemical values of Patient 2

| Day | 1 | 7 | 15 |
|---|---|---|---|
| Calcium (mg. %) | 10.0 | 10.1 | 10.2 |
| Phosphorous (mg. %) | 5.0 | 5.1 | |
| Fasting blood sugar (mg. %) | 86 | 91 | 82 |
| Alkaline phosphatase (Shirowara units) | 9.0 | 7.4 | 6.8 |
| Total protein (Gm. %) | 7.0 | | |
| A. | 3.7 | | |
| G. | 3.3 | | |
| Blood urea nitrogen (mg. %) | 19 | 15 | 16 |
| Sodium (mEq./L.) | 140 | 143 | |
| Potassium (mEq./L.) | 4.4 | 4.2 | |
| Chlorine (mEq./L.) | 106 | | |
| Nonesterified fatty acids (mEq. of palmitic acid) | 1,280 | 1,199 | |
| Cholesterol (mg. %) | | 165 | |
| Treatment | None | 5 mg. HGH (third day) | 5 mg. HGH (tenth day) |

normally functioning pituitary mechanism or to the inability of the end organs to respond; however, until more basic information is available it is unwise to theorize.

The treatment failed to alter the child's increment of growth (Figs. 6 and 7), but because of the shortness of the period of therapy more prolonged studies should be made.

Human growth hormone has been reported by Crigler and co-workers[17] to be ineffective in idiopathic hypoglycemia and by Grumbach[18] to be ineffective in normal premature infants. Grumbach's studies are not yet available in detail. The dwarfing associated with gonadal dysgenesis (Turner's syndrome) has been under investigation by Hutchings[19] and dramatic results have not been seen initially.

## SUMMARY

A "hypopituitary" dwarf was treated during a 10-month period with human growth hormone (Raben[12]); there was a resultant spurt in growth associated with relatively slight, if any, maturation in body proportions and skeleton. Radiologic evidence of widening of the epiphyseal plates was noted.

A constitutional or primordial dwarf was treated with similar amounts of growth hormone and failed to show significant reduction in urine nitrogen, changes in blood chemistries, or a spurt in linear growth.

Neither patient had any apparent adverse reaction to the growth hormone.

## REFERENCES

1. Beck, J. C., McGarry, E. E., Dyrenfurth, I., and Venning, E. H.: Metabolic Effects of Human and Monkey Growth Hormone in Man, Science **125:** 884, 1957.
2. Hutchings, J. S., Escamilla, R. F., Deamer, W. C., and Li, C. H.: Metabolic Changes Produced by Human Growth Hormone (Li) in a Pituitary Dwarf, J. Clin. Endocrinol. **19:** 759, 1959.
3. Raben, M. S.: Treatment of a Pituitary Dwarf With Human Growth Hormone (Letter to the Editor), J. Clin. Endocrinol. **18:** 901, 1958.
4. Henneman, P. H., Moldawer, M., Carroll, Evelyn L., Mitchell, Mary, and Waddell, Mary J.: Human Growth Hormone: Prolonged Administration of Moderate Doses in Three Patients With Panhypopituitarism, Abstract No. 40, Program of 40th Meeting of the Endocrine Society, San Francisco, June 20, 1958.
5. Ikkos, D., Luft, R., and Gemzell, C. A.: The Effect of Human Growth Hormone in Man, Lancet **1:** 720, 1958.
6. Panel Appointed by the Clinical Endocrinology Committee of the Medical Research Council: The Effectiveness in Man of Human Growth Hormone, Lancet **1:** 7, 1959.
7. Shepard, T. H., Nielsen, R. I., Johnson, Mary Lou, and Bernstein, Nan: Human Growth Hormone. I. Metabolic Balance Studies Carried Out in a Hypopituitary Child. A. M. A. J. Dis. Child. **99:** 90, 1960.
8. Thuline, H., Shepard, T. H., and Creighton, S. A.: Chromosomal Sex Test: Pediatric Applications, A. M. A. J. Dis. Child. **94:** 130, 1957.
9. Simpson, Miriam E., Asling, C. W., and Evans, H. M.: Some Endocrine Influences on Skeletal Growth and Maturation, Yale J. Biol. & Med. **23:** 1, 1950.
10. Stuart, H. C., and Meredith, H. V.: Use of Body Measurements in the School Health Program, Am. J. Public Health **36:** 1366, 1946.
11. Saito, K.: Physical and Development State of Babies and Children: Survey of 1950, National Public Health Institute of Japan, 1956.
12. Raben, M. S., in Pincus, Gregory: Human Growth Hormone, Recent Progress in Hormone Research, New York, 1959, Academic Press, Inc., vol. 15, p. 76.
13. Raben, M. S., in Pincus, Gregory: Human Growth Hormone, Recent Progress in Hormone Research, New York, 1959, Academic Press, Inc., vol. 15, p. 91.
14. McCune, D. J.: Dwarfism, Clinics **2:** 380, 1943 to 1944.
15. Silver, H. K., Kiyasu, W., George, J., Deamer, W. C.: Syndrome of Congenital Hemihypertrophy, Shortness of Stature and Elevated Urinary Gonadotropins, Pediatrics **12:** 368, 1953.
16. Grumbach, M. M.: Personal communication.
17. Crigler, J. F., Knapp, J. A., Chagnon, Jean: Observations on the Metabolic Effects of Glucagon and Growth Hormone (Human and Beef) in an Infant With Idiopathic Hypoglycemia and Hyperinsulinism, A. M. A. J. Dis. Child. **96:** 432, 1958.
18. Grumbach, M. M., in Pincus, Gregrory: Human Growth Hormone, Recent Progress in Hormone Research, New York, 1959, Academic Press, Inc., vol. 15, p. 110.
19. Hutchings, J.: Personal communication.

# *Effect of certain nor-steroids on creatine excretion*

**Walter Fleischmann, M.D.,* Carl Ferrell, M.S., Robert L. Fischer, Ph.D., and James N. Etteldorf, M.D.**

MEMPHIS, TENN.

WILKINS and his co-workers[1] reported in 1941 that administration of methyltestosterone induces creatinuria in dwarfed children of both sexes. The effect was observed after a latent period of 4 to 16 days and was continued as long as the drug was administered. Further studies[2] showed that this effect was specific for androgens with a methyl group attached to the 17-carbon atom. For the purposes of the present investigation it is of interest that androstenolone (testosterone) and ethylandrostenolone (ethyltestosterone) did not induce creatinuria in contrast to the effect of methyl androstenolone (methyltestosterone). Creatinuria due to methyltestosterone was also observed in healthy young men.[3]

The mechanism of this effect was elucidated by Hoberman, Sims, and Engstrom,[4] who presented evidence by means of isotope methods that methyltestosterone induces an increase in the rate of synthesis of the creatine precursor, guanidoacetic acid. The increased excretion of guanidoacetic acid following the administration of methyltestosterone[4, 5] is consistent with the hypothesis that methyltestosterone increases the formation of creatine specifically by its synthesis of guanidoacetic acid.

As compounds of the nor-steroid series became available it seemed of interest to study their possible effect on creatine excretion. We expected that normethandrolone (methyl nortestosterone)* would induce creatinuria, but that norethandrolone (ethylnortestosterone)* would be without effect on creatine in analogy to the behavior of the testosterone series cited above. To our surprise creatinuria was induced by both compounds.

## METHODS AND RESULTS

The patients included in this study were hospitalized in the West Tennessee Tuberculosis Hospital for treatment of primary tuberculosis. Antibacterial therapy had been

*From the Research Laboratory of the Division of Pediatrics and Division of Chemistry, University of Tennessee, Frank T. Tobey Memorial Children's Hospital, and the West Tennessee Tuberculosis Hospital, Memphis, Tenn.*

*This work was made possible, in part, by a grant in aid from the National Heart Institute, National Institutes of Health, Project H454C9, and by a grant from the G. D. Searle Company of Chicago.*

**Present address, Chief, Laboratory Service, Veterans Administration Center, Mountain Home, Tenn.*

*Supplied by the G. D. Searle Company of Chicago, Ill. Ethylnortestosterone was supplied under the trade name Nilevar.

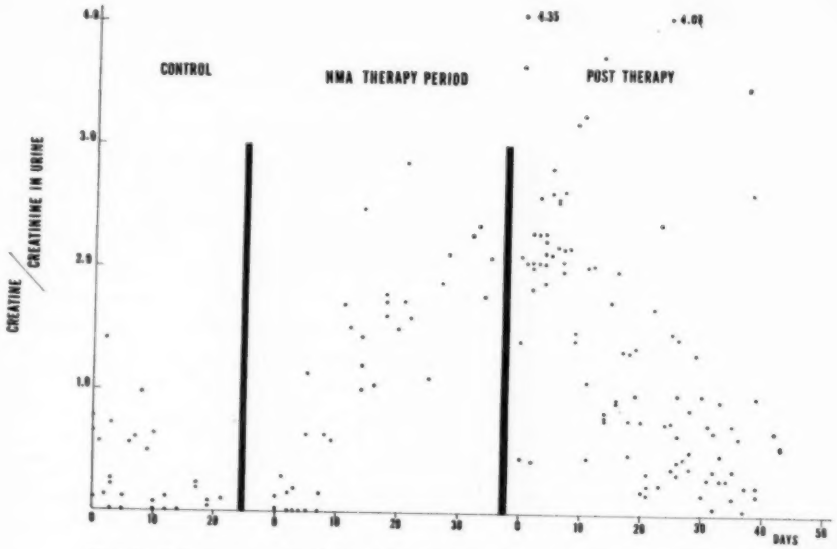



**Fig. 1.** Urinary creatine/creatinine ratios of 5 patients treated with normethandrolone (NMA). Each point represents the ratio for a 5-day period computed from individual curves.

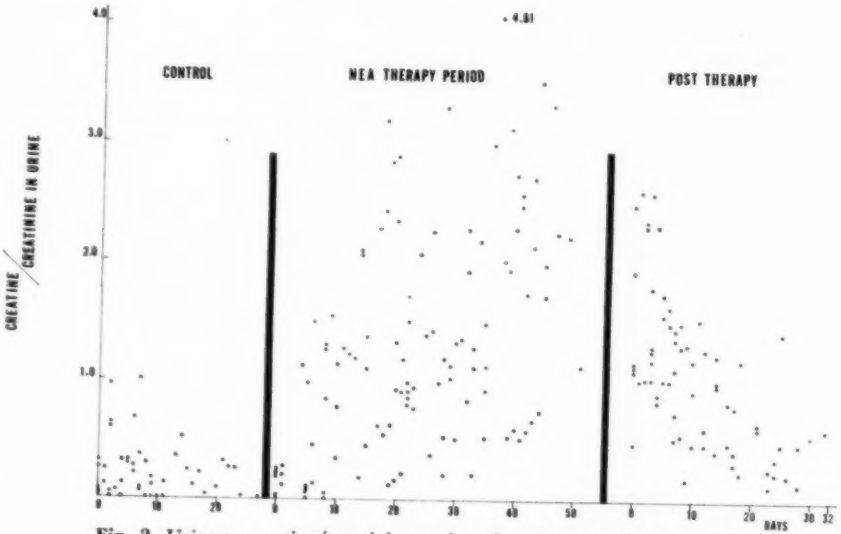



**Fig. 2.** Urinary creatine/creatinine ratios of patients treated with norethandrolone (NEA). Each point represents the ratio for a 5-day period computed from individual curves.

completed or else therapy was safely delayed until the termination of the studies to be reported. In the latter cases, the patients' welfare was not compromised.

The ages of the patients ranged from 5 to 12 years. There were 7 females and 2 males. Two patients, Nos. 2 and 3, received norethandrolone and normethandrolone dur-

**Table I.** Urinary creatine/creatinine ratios in patients treated with normethandrolone (NMA)

| Patient | Age | Sex | Dosage (mg./Kg./day) | Urinary creatine/creatinine | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Control period | | Therapy period | | Posttherapy period | |
| | | | | Range | Mean | Range | Mean | Range | Mean |
| 1 | 12 | M | 0.6 | 0.00-0.20 | 0.04 | 0.00-1.50 | 0.56 | 0.04-2.16 | 0.74 |
| 2 | 10 | F | 1.0 | 0.09-0.97 | 0.37 | 0.12-2.85 | 1.54 | 0.19-3.65 | 1.81 |
| 3 | 9 | F | 1.0 | 0.13-0.26 | 0.18 | 0.64-2.34 | 1.50 | 0.23-2.54 | 1.46 |
| 4 | 7 | F | 1.2 | 0.56-1.42 | 0.75 | 0.15-0.28 | 0.21 | 0.35-4.35 | 1.89 |
| 9 | 10 | M | 0.6 | 0.05-0.22 | 0.10 | 0.00-1.60 | 1.43 | 0.22-2.13 | 0.75 |

**Table II.** Urinary creatine/creatinine ratios in patients treated with norethandrolone (NEA)

| Patient | Age | Sex | Dosage (mg./Kg./day) | Urinary creatine/creatinine | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Control period | | Therapy period | | Posttherapy period | |
| | | | | Range | Mean | Range | Mean | Range | Mean |
| 2 | 10 | F | 1.0 | 0.09-0.97 | 0.37 | 0.27-4.61 | 1.70 | 0.26-2.47 | 1.04 |
| 3 | 9 | F | 1.0 | 0.03-1.01 | 0.46 | 0.02-2.98 | 1.51 | 0.21-2.35 | 0.93 |
| 5 | 12 | F | 1.0 | 0.00-0.38 | 0.07 | 0.00-1.97 | 0.51 | 0.19-1.40 | 0.68 |
| 6 | 5 | F | 1.0 | 0.00-0.32 | 0.10 | 0.00-3.50 | 2.10 | 0.13-2.60 | 1.50 |
| 7 | 6 | F | 1.0 | 0.06-0.65 | 0.23 | 0.11-2.24 | 0.86 | 0.61-1.26 | 0.98 |
| 8 | 9 | F | 1.0 | 0.01-0.68 | 0.23 | 0.05-1.31 | 0.52 | 0.42-1.29 | 0.78 |

**Table III.** Urinary guanidoacetic acid/creatinine ratios in patients treated with normethandrolone (NMA) and norethandrolone (NEA)

| Patient | Age | Sex | Drug | Dosage (mg./Kg./day) | Urinary guanidoacetic acid/creatinine | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Control period | | Therapy period | | Posttherapy period | |
| | | | | | Range | Mean | Range | Mean | Range | Mean |
| 1 | 12 | M | NMA | 0.6 | 0.057-0.118 | 0.089 | 0.018-0.154 | 0.098 | 0.066-0.201 | 0.101 |
| 4 | 7 | F | NMA | 1.2 | 0.053-0.153 | 0.087 | 0.033-0.056 | 0.042 | 0.017-0.296 | 0.108 |
| 7 | 6 | F | NEA | 1.0 | 0.013-0.058 | 0.039 | 0.044-0.109 | 0.069 | 0.044-0.093 | 0.065 |
| 8 | 9 | F | NEA | 1.0 | 0.016-0.061 | 0.040 | 0.025-0.101 | 0.067 | 0.031-0.127 | 0.071 |
| 9 | 10 | M | NMA | 0.6 | 0.059-0.113 | 0.090 | 0.028-0.251 | 0.105 | 0.058-0.239 | 0.127 |

ing different experimental periods. Four patients, Nos. 5, 6, 7, and 8, received only norethandrolone and 3 patients, Nos. 1, 4, and 9 received only normethandrolone. The dose of steroids ranged from 0.6 to 1.2 mg. per kilogram per day with a total dose ranging from 20 to 30 mg. per day. During the experimental period, all patients gained weight; the range in weight gain was 14 to 20 per cent when compared to the weight during the control period. The weight remained essentially stationary during the period following therapy.

Urine specimens, usually 24-hour collections, were collected for analysis 3 times per week. Control periods ranged from 2 to 27 days and the experimental period during which steroid was administered ranged from 22 to 51 days. Observations continued for 14 to 43 days after cessation of treatment.

Urinary creatine and creatinine levels were determined by the method of Folin, urinary guanidoacetic acid levels by the method of Hoberman.[7] Because of inability to control accurately the duration of urine collections at all times, the data are pre-

sented as creatine/creatinine and guanidoacetic acid/creatinine ratios, thus eliminating the influence of possible irregularities in urine collections.

Both normethandrolone and norethandrolone induced a definite and reversible creatinuria (Tables I and II, Figs. 1, 2, 3, and 4). The increase in the creatine/creatinine ratio during the control period when compared with the ratio obtained during the period of treatment or the period immediately following treatment is significant for both compounds. Treatment with normethandrolone in 3 patients (1, 4, and 9) resulted in an increase in excretion of guanidoacetic acid as indicated by an increased guanidoacetic acid/creatinine ratio (Table III). Treatment with norethandrolone also resulted in an increase in guanidoacetic acid/creatinine ratio (Table III). The absolute increase in this ratio will be observed to be approximately the same following the administration of both compounds.

## DISCUSSION

Creatinuria due to norethandrolone has recently been reported by Stokes and colleagues,[8] while the patient was on a tube-feeding diet containing small amounts of creatine. Newman[9] observed suppression of creatinuria in a patient with thyrotoxicosis during administration of norethandrolone. The rise in excretion of guanidoacetic acid after administration of normethandrolone is comparable to the rise in excretion of this compound due to methyltestosterone reported in the literature.[4] Our data indicate that norethandrolone also causes an increase in creatine excretion and therefore differs from ethyltestosterone in that the latter did not result in creatinuria.[2] These results cast certain doubts upon the essential influence of the methyl group on creatine excretion following the use of certain androgens and also call to attention the possible role of spacial configuration in the "nor" compounds.

Although it is possible that creatinuria resulting from normethandrolone and creatinuria from norethandrolone are induced by different mechanisms, our limited data do not permit this conclusion. Further investigations of these compounds with regard to the etiology of the creatinuria are indicated. The mechanisms of creatinuria produced by other androgens with a methyl group attached to the carbon in the 17-position has

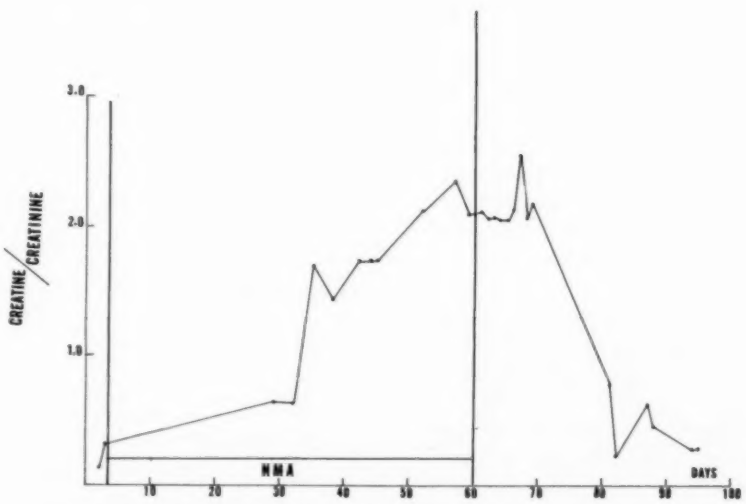



**Fig. 3. Urinary creatine/creatinine ratios in a 9-year-old female patient, No. 3, receiving normethandrolone (NMA), 1 mg. per kilogram per day for 57 days.**

Fig. 4. Urinary creatine/creatinine ratios in patient No. 3, receiving norethandrolone (NEA), 1 mg. per kilogram per day for 47 days.

been attributed to an increase in the precursor, guanidoacetic acid.[4] Stokes and coauthors[8] suggest that norethandrolone increases both the synthesis and the storage of creatine in the muscle. These investigators did not study the methyl compound. Creatinuria due to a decreased ability of the renal tubule to reabsorb creatine is a possible explanation. This mechanism has been shown to be operative in the creatinuria caused by the administration of desoxycorticosterone.[10]

## SUMMARY

1. Urinary creatine excretion was studied in 5 children following the administration of normethandrolone and 6 children following the administration of norethandrolone.

2. Both normethandrolone and norethandrolone induce reversible creatinuria in children.

3. Administration of normethandrolone and norethandrolone is associated with a slight increase in urinary excretion of the creatine precursor, guanidoacetic acid.

4. The mechanism of action of these compounds on creatine metabolism is possibly not identical with that of methyltestosterone and requires additional study in order to elucidate the mechanism of action.

The authors wish to thank Dr. E. P. Bowerman and the medical and nursing staff of the West Tennessee Tuberculosis Hospital for their cooperation and assistance.

## REFERENCES

1. Wilkins, L., Fleischmann, W., and Howard, J. E.: Creatinuria Induced by Methyltestosterone in the Treatment of Dwarfed Boys and Girls, Bull. Johns Hopkins Hosp. **69:** 493, 1941.
2. Wilkins, L., and Fleischmann, W.: Studies on the Creatinuria Due to Methylated Steroids, J. Clin. Invest. **24:** 21, 1945.
3. Samuels, L. T., Henschel, A. F., and Keys, A.: Influence of Methyl Testosterone on Muscular Work and Creatine Metabolism in Normal Young Men, J. Clin. Endocrinol. **2:** 649, 1942.
4. Hoberman, H. D., Sims, E. A. H., and Engstrom, W. W.: The Effect of Methyltestosterone on the Rate of Synthesis of Creatine, J. Biol. Chem. **173:** 111, 1947.
5. Hoagland, C. L., Shank, R. E., and Gildea H.: Effect of Testosterone Propionate and Methyltestosterone in Progressive Muscular Dystrophy, Proc. Soc. Exper. Biol. & Med. **55:** 49, 1944.
6. Hawk, P. B., Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, ed. 13, New York, 1954, McGraw-Hill Book Company, Inc., p. 903.
7. Hoberman, H. D.: The Determination of Guanidoacetic Acid and Arginine in Human Urine and Serum, J. Biol. Chem. **167:** 721, 1947.

8. Stokes, P. E., Horvith, M., Pennington, T. G., and Clarkson, B.: 17-Alpha-Ethyl-19-Nortestosterone as an Anabolic Agent and Its Effect on Creatine Metabolism and Vaginal Cytology in Humans, Metabolism **8**: 709, 1959.
9. Newman, E. V.: Some Medical Aspects of Anabolic Agents, in Proceedings of a Conference on the Clinical Use of Anabolic Agents, Chicago, April, 1956, Searle Research Laboratories, pp. 32-35.
10. Zierler, K. L., Folk, B. P., Magladery, J. W., and Lilienthal, J. L., Jr.: On Creatinuria in Man. Roles of Renal Tubule and of Muscle Mass, Bull. Johns Hopkins Hosp. **85**: 370, 1949.

# *A case of proved adrenocorticotropin deficiency*

**William W. Cleveland, M.D., Orville C. Green, M.D.,* and**

**Claude J. Migeon, M.D.****

BALTIMORE, MD.

PITUITARY deficiency in childhood is a comparatively rare disorder and the diagnosis depends upon indirect evidence of target gland hypofunction. If sexual infantilism persists beyond the expected age of puberty, the diagnosis may be considered more certain, since failure of sexual maturation is a constant feature of generalized pituitary deficiency. The problems related to this disturbance and its diagnosis have been reviewed recently.[1]

It is less common to encounter a case where evidence appears to indicate an isolated deficiency of a single tropic hormone of the pituitary. Specific deficiency of thyrotropic hormone, leading to hypothyroidism, may occur.[2] It is also recognized that sexual infantilism with eunuchoid body build may result from an apparent specific defect in elaboration of gonadotropic hormone. The proof of a specific defect of growth hormone, leading only to short stature, must await satisfactory methods of analysis for this hormone. There is one previously reported case of adrenocortical insufficiency due to a suspected isolated deficiency of adrenal corticotropin.[3] In this clinic, we have recently examined an adolescent girl whose clinical appearance suggested a defect of corticotropin without panhypopituitarism, and subsequent studies seem to substantiate this diagnosis. The findings in this case will be presented in detail.

*From the Endocrine Division of the Department of Pediatrics, The Johns Hopkins Hospital and The Johns Hopkins Medical School.*

*This work was made possible by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health (A-180), United States Public Health Service.*

**Recipient of a Research Fellowship of the United States Public Health Service.*

***Address, Department of Pediatrics, The Johns Hopkins Hospital, Baltimore 5, Md.*

## CASE REPORT

P. W. (JHH No. 827169), a 15½-year-old girl visited the Endocrine Clinic for the first time on Nov. 10, 1958, for evaluation of her short stature. Although no measurements had been kept, she was reported to have been small all her life, and growth delay had become increasingly evident in the past few years. The parents stated that she had been anorexic throughout childhood and at the age of 6 years had been treated for anemia with injections of liver extract. However, there had been no serious illnesses and previous examinations had disclosed no evidence of systemic disease. She had remained alert, intelligent, and reasonably active. There was no history of significant headaches, visual disturbances, polydipsia, or polyuria. Breast development had begun one year previously, with progressive enlargement. Menarche had not yet occurred, and neither axillary nor pubic hair had been

noted. Approximately one month prior to this visit, her local physician had performed tests which showed urinary 17-ketosteroids of 0.6 mg. per day and normal skull x-rays. No therapy was started and she was referred to this clinic for further study.

Upon examination, her height was 55¼ inches (height age 11 years) and her weight was 63 pounds. Blood pressure was 100/70 in both supine and sitting positions. The general appearance was that of a small, pale, slender girl with immature facies appearing much younger than the actual age (Fig. 1). There was no increased skin pigmentation; in fact, the skin was paler than usual. Breasts were moderately well developed measuring 6 to 7 cm. in diameter. There was no axillary or pubic hair. The genitals were not considered abnormal, and there was definite pubertal enlargement of the labia minora. On rectal examination, the body of the uterus was palpable although the cervix could not be definitely felt. No masses were found in the inguinal or pelvic areas.

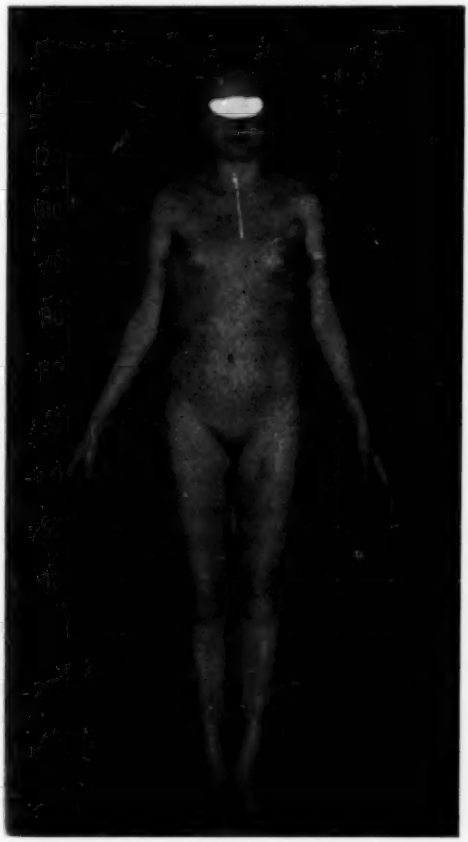


**Fig. 1.** Patient (P. W.) prior to the beginning of therapy.

Extensive clinical and laboratory studies were made during repeated visits throughout a period of five months. In this interval, her condition remained unchanged. The results of these examinations are listed below.

**Laboratory data.** *Radiological.* Osseous development had progressed to a level of 14 to 15 years, by estimation of multiple centers of ossification and fusion. There were no abnormalities noted on skull x-rays.

*Nuclear chromatin pattern.* The buccal smear was chromatin positive ("female").

*Hematologic.* The hemoglobin was 11.2 Gm. per cent and the hematocrit 35.6 per cent.

*Urinalysis.* Urinalysis was normal.

*Vaginal smear.* This showed cornified and precornified epithelial cells with pyknotic nuclei, indicating estrogen effect.

| *Chemical* | *11/10/58* | *12/11/58* | |
|---|---|---|---|
| Protein-bound iodine | 6.0 | 5.1 | μg % |
| Cholesterol | 308 | 330 | mg.% |
| Serum sodium | 148 | 158 | mEq./L. |
| Serum potassium | 4.7 | 4.5 | mEq./L. |
| Serum chloride | 104 | 100.5 | mEq./L. |
| Carbon dioxide combining power | 25.0 | 26.2 | mEq./L. |
| Urea nitrogen | 17 | | mg.% |
| Fasting glucose | | 72 | mg.% |
| Calcium | | 11.6 | mg.% |
| Phosphorus | | 2.4 | mg.% |
| Alkaline phosphatase | | 6.8 | Bodansky units |

*Gonadotropin assay.* (Method of Bradbury, Brown, and Brown.[4]) This was positive at 6.6 m.u., negative at higher dilutions (normal: 6.6 to 52.6 m.u.).

*Ability to excrete a water load.* The patient ingested 20 ml. of water per kilogram of body weight, at 9:00 A.M. The total 4-

hour excretion was 18 per cent (normal: greater than 65 per cent). The total excretion after 3 days of therapy with ACTH was 69 per cent.

*Studies of adrenocortical function.* Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels.[8] Total urinary 17-hydroxycorticosteroids were determined by the method of Glenn and Nelson.[6] For the measurement of the individual urinary 17-hydroxycorticosteroids the extract obtained after chromatography on Florisil columns was divided into two equal portions and each portion applied on Whatman No. 2 paper. Chromatography was carried out with the system of solvents, benzene:methanol:water (100:55:45) of Bush.[7] Reference steroids were chromatographed alongside the extracts. The paper strip corresponding to one of the two aliquots was then stained with blue tetrazolium and the parallel areas of the unstained strip corresponding to the spots obtained with blue tetrazolium were cut out and eluted by the wick technique of Bush, using ethanol. The ethanol eluate was evaporated to dryness and divided into equal portions; one portion was used as a blank with ethanol-sulfuric acid and the other portion was treated with the Porter-Silber reagent.[8] When the aliquots applied to paper are kept in a range below 40 μg, recoveries of standards average 85 per cent.

Urinary 17-ketosteroids were determined by a modification of the method of Callow, Callow, and Emmens.[9]

The control levels of 17-hydroxycorticosteroids and 17-ketosteroids were found to be low (Table I). The ability of the adrenal cortex to produce increased amounts of steroids under the influence of ACTH was tested by the administration of Acthar-gel (Armour), 20 units twice a day for 3 days (Feb. 24 to 26, 1959). There was a good response to this stimulation with a rise of levels of 17-hydroxycorticosteroids from a control value of 0.8 to 11.9 mg. per 24 hours on the third day.

Liddle and associates[10, 11] have suggested that the compound, 2-methyl-1, 2-bis-(3-pyridyl)-1-propanone (SU-4885), may be utilized to test pituitary ability to elaborate ACTH. This drug was administered to the patient and to a control subject (W. H.) of similar weight at the dosage of 500 mg. every 6 hours for 48 hours. The results of these studies are shown in Table I and Fig. 2. It will be noted that the patient showed no significant rise in levels of total 17-hydroxycorticosteroids after 48 hours of treatment with SU-4885, although it should be pointed out that the small increase of steroids produced during this period was almost entirely "tetrahydro S." In contrast, the control subject showed a definite response in total corticosteroid excretion, the majority of which was "tetrahydro S."

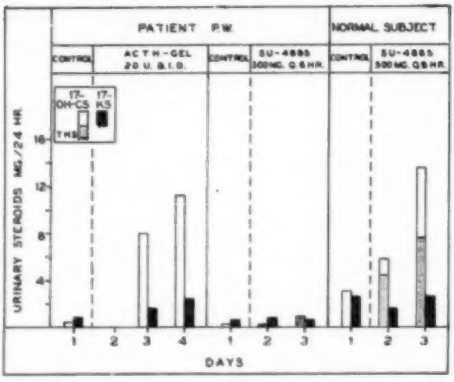



**Fig. 2.** Steroid responses of patient and a normal control subject.

*Results of treatment.* Although it would have been of interest to treat the patient with ACTH, this was not considered practical. It was decided, therefore, to give substitution therapy with methyltestosterone and cortisone. In order to observe the separate effects of these drugs she was given methyltestosterone alone, 20 mg. daily for 3 months, at which time it was reduced to a dose of 10 mg. daily. It is planned to add therapy with cortisone at some future time. There has been a definite response to androgenic treatment with increased appetite and vigor. After 6 months of treatment a growth of pubic hair has appeared and she has grown 1½ inches and gained 17 pounds. Menstruation has not yet occurred.

## DISCUSSION

The general appearance of this patient at first suggested that of a child with hypopituitarism, manifesting rather marked dwarfing and a somewhat characteristic immaturity of the facies. This impression was also borne out by the absence of sexual hair. However, the finding of well-developed breasts and estrinized labia minora is most unusual in panhypopituitarism as there is usually a deficiency of gonadotropin. Feminization without development of sexual hair may also be found in that type of male pseudohermaphroditism with testicular feminization, where female secondary sexual characteristics develop at puberty. It has been recognized that approximately one-third of such patients fail to develop sexual hair. These patients are not stunted in growth, however, do not have a uterus, and often have palpable gonads in the inguinal or labial regions. This possibility was excluded by the finding of a chromatin-positive nuclear pattern in the buccal smear. Another diagnostic possibility included consideration of some end-organ defect accounting for failure of development of sexual hair, but the response to treatment with methyltestosterone proved this not to be the case. It was also considered possible that this case simply represented a delay of adolescence with the very unlikely occurrence of breast development prior to the appearance of sexual hair.

The most reasonable hypothesis seemed to be that this child had some deficiency of adrenal hormones. There was no excessive pigmentation nor was there any history to suggest an electrolyte disturbance or other symptoms of adrenal cortical failure. The combination of all these findings suggested that partial impairment of adrenal function might be present as a result of inadequate elaboration of pituitary adrenocorticotropin. Studies of adrenocortical function were undertaken to test this hypothesis.

Laboratory data subsequently obtained confirmed the fact that generalized deficiency of pituitary tropic hormones was not present. Urinary tests for gonadotropins were positive and the protein-bound iodine levels in the serum were normal on two occasions. Inadequate adrenal-cortical function was demonstrated by repeated findings of impaired ability to excrete a water load and low values for the urinary 17-ketosteroids and 17-hydroxycorticosteroids. Plasma 17-hydroxycorticosteroids were likewise low. Proof that the adrenal glands were capable of functioning was obtained by administering Acthar-gel intramuscularly for three days and finding significant increases in urinary steroids with correction of the excretion of the water load.

**Table I.** Steroids in plasma and urine

| Date | Therapy | Urinary 17-KS | Urinary 17-OHCS | | Plasma 17-OHCS (μg %) |
|---|---|---|---|---|---|
| | | | Total | "Tetra-S" | |
| *Patient P. W.* | | | | | |
| 11/12/58 | None | 1.2 | | | |
| 12/ 7/58 | None | 0.3 | 0.7 | 0. | |
| 2/23/59 | None | 0.8 | 0.3 | | 3.1 |
| 2/24/59 | Acthar-gel | — | — | | |
| 2/25/59 | Acthar-gel | 1.7 | 7.9 | 0. | |
| 2/26/59 | Acthar-gel | 2.7 | 11.2 | 0. | 29.7 |
| 3/16/59 | Control | 0.5 | 0.2 | 0. | |
| 3/17/59 | SU-4885 | 0.7 | 0.3 | 0.3 | |
| 3/18/59 | SU-4885 | 0.6 | 0.9 | 0.8 | |
| *Normal subject W. H.* | | | | | |
| 4/ 2/59 | Control | 2.55 | 3.1 | 0. | |
| 4/ 3/59 | SU-4885 | 1.94 | 5.7 | 4.3 | |
| 4/ 4/59 | SU-4885 | 2.51 | 13.4 | 7.7 | |

It had been reported previously by Liddle[10-11] and Jenkins[12] that pituitary "reserve" might be tested by the administration of SU-4885. This drug inhibits the adrenal enzyme, 11-beta-hydroxylase, thus inhibiting the synthesis of cortisol. With the resultant drop in circulating cortisol, a normal individual will respond with an increased production of adrenocorticotropin in a homeostatic attempt to stimulate production of more cortisol. However, because of the inhibited enzymatic pathway, the net result is an increased production of one of the intermediates in the biosynthesis of cortisol. Substance S of Reichstein (17, 21-dihydroxy-pregnene-3,20-dione) may be measured either in the blood or in the urine as its excretory product "tetrahydro-S." Since substance S is relatively inert biologically and does not inhibit ACTH production there continues to be an elaboration of corticotropin in increased amounts with a consequent increase in total output of steroids, composed largely of substance S. It has also been shown that hypophysectomized patients do not demonstrate this response. A failure of response in this patient is evident from the data obtained.

A limited capacity to produce pituitary adrenocorticotropin might explain many of the features of this unusual clinical picture. It would be unwarranted to say that this case represents an instance of isolated deficiency of corticotropin since the patient's short stature may have been due to deficiency of growth hormone. However, the effects upon growth of long-standing, untreated insufficiency of adrenal hormones is not clear and may be significant.

It is of interest to compare this case with the one reported by Steinberg, Schechter, and Segal.[3] In their case generalized deep pigmentation of skin and mucus membranes was noted, whereas our patient showed no increased pigmentation but in contrast appeared somewhat pale in spite of a normal level of hemoglobin. Although no exact measurements of this previous patient are given it would appear from the photographs that she is approximately 56 to 57 inches tall as compared with our patient's height of 55¼ inches. There is no statement in their case report as to the presence or absence of sexual hair. The response in their case to ACTH stimulation seems well documented although, of course, the assay of ACTH elaboration by the indirect technique of response to SU-4885 was not available at that time.

## SUMMARY

A case is presented of proved deficiency of pituitary adrenocorticotropin with apparently adequate elaboration of pituitary thyrotropic and gonadotropic hormones. In the absence of a method for specific testing, the status of growth hormone elaboration remains obscure.

The authors are grateful to Dr. Lawson Wilkins for advice regarding the management of this case and the preparation of this report. The SU-4885 was generously supplied by Dr. C. H. Sullivan of Ciba Pharmaceutical Products, Inc.

## REFERENCES

1. Martin, M. M., and Wilkins, L.: Pituitary Dwarfism: Diagnosis and Treatment, J. Clin. Endocrinol. **18:** 679, 1958.
2. Querido, A., and Stanbury, J. B.: The Response of the Thyroid Gland to Thyrotropic Hormone as an Aid in the Differential Diagnosis of Primary and Secondary Hypothyroidism, J. Clin. Endocrinol. **10:** 1192, 1950.
3. Steinberg, A., Schechter, F. R., and Segal, H. I.: True Pituitary Addison's Disease—a Pituitary Unitropic Deficiency, J. Clin. Endocrinol. **14:** 1519, 1954.
4. Bradbury, J. T., Brown, E. S., and Brown, W. E.: Absorption of Urinary Gonadotropins on Kaolin, Proc. Soc. Exper. Biol. & Med. **71:** 228, 1949.
5. Nelson, D. H., and Samuels, L. T.: A Method for the Determination of 17-Hydroxycorticosteroids in Blood: 17-Hydroxycorticosterone in the Peripheral Circulation, J. Clin. Endocrinol. **19:** 519, 1952.
6. Glenn, E. M., and Nelson, D. H.: Chemical Method for the Determination of 17-Hydroxycorticosteroids and 17-Ketosteroids in Urine Following Hydrolysis With β-Glucuronidase, J. Clin. Endocrinol. **13:** 911, 1953.
7. Bush, I. G.: Methods of Paper Chromatography of Steroids Applicable to the Study of Steroids in Mammalian Blood and Tissue, Biochem. J. **50:** 370, 1952.
8. Porter, C. C., and Silber, R. H.: A Quantitative Color Reaction for Cortisone and Related 17,21-Dehydroxy-20-Ketosteroids, J. Biol. Chem. **185:** 201, 1950.

9. Callow, H. H., Callow, R. K., and Emmens, C. W.: Colorimetric Determination of Substances Containing the Grouping $CH_2CO$ in Urine Extracts as Indication of Androgen Content, Biochem. J. **32:** 1312, 1938.
10. Liddle, G. W., Island, D., Lance, E. M., and Harris, A. P.: Alterations of Adrenal Steroid Patterns in Man Resulting From Treatment With a Chemical Inhibitor of 11$\beta$-Hydroxylation, J. Clin. Endocrinol. **18:** 906, 1958.
11. Liddle, G. W., Estop, H. L., Kendall, J. W., Jr., Williams, W. C., Jr., and Townes, A. W.: Clinical Application of a New Test of Pituitary Reserve, J. Clin. Endocrinol. & Metab. **19:** 875, 1959.
12. Jenkins, J. S., Meakins, J. W., Nelson, D. H., and Thorn, G. W.: Inhibition of Adrenal Steroid 11-Oxygenation in the Dog, Science **128:** 478, 1958.

# *Acromegaly and gigantism in a boy: comparison with 3 overgrown nonacromegalic children*

**Salvador F. de Majo, M.D.,* and Arturo Oñativia, M.D.**

BUENOS AIRES AND SALTA, ARGENTINA

MARIE[1] described acromegaly in adult patients for the first time in 1886, concluding that, "There is a disease characterized mainly by hypertrophy of feet, hands and face that we propose to call acromegaly, that is to say, hypertrophy of the extremities (not only because the extremities are affected during the whole course of the disease, but because the increase of their size is an initial phenomenon which is the most characteristic feature of this disorder)."

This increase in size is due fundamentally to 2 characteristic and generalized manifestations of the disease, namely the thickening of the soft tissues (cutaneous and subcutaneous alterations[2]) and the modification of osseous structure leading to thickening of the bones.

The existence of this clinical feature has rarely been noted to occur in children. Following the descriptions of Brissaud and Meigge[3] it generally has been accepted that gigantism is found in childhood and that acromegaly appears afterwards or in connection with the closure of the epiphyseal cartilages. This concept would suggest that the increase of volume of the extremities (thickening of the soft tissues and bones) does not take place while longitudinal growth is possible.

The patient described below, studied in comparison with other overgrown children, does not seem to confirm this concept, which suggests interesting clinical and pathologic considerations.

## CASE REPORT

O. P., a boy of 9 years 4 months, came to the clinic complaining of persistent cephalalgia and rapid growth.

He had been full term at birth and weighed 2,960 grams. He had always been taller than other children his age and in the preceding 1½ years had grown more rapidly. According to his parents, he had grown 20 cm. in that period and had shown a disproportionate development of hands and feet. Starting at the same time, the

*From 3ª Cátedra de Pediatría de la Universidad de Buenos Aires, Casa Cuna, and Instituto de Endocrinología, Salta, Argentina.*

*This case was presented to the Pan American Congress of Endocrinology, Buenos Aires, November, 1957.*

**Address, 3ª Cátedra de Pediatría de la Universidad de Buenos Aires, Casa Cuna, Buenos Aires, Argentina.*

patient had suffered headaches, particularly on the right side, 2 or 3 times a day. When the boy was seen the cephalalgia was constant, and he responded poorly to analgesics. There was no nausea, vomiting, or dizziness. He showed some apathy and irritability, although intelligence was normal. He did not complain of visual disturbances, and no polyuria was reported.

His parents were of normal height and had delicate features. There was no history of tall persons or endocrine disorders in the family. There were no siblings.

**Physical findings.** His height was 60¼ inches (normal height for a person of 13 years 8 months) and the upper/lower ratio was 0.99. He weighed 52 kilograms.

He had a large, oval, coarse face. Thickness of his features was an outstanding finding, most noticeable in the ears, nose, lips, and tongue. The mandible was rather large and the superior incisors widely spaced. The hands and feet were broad. All this gave the general appearance of acromegaly.

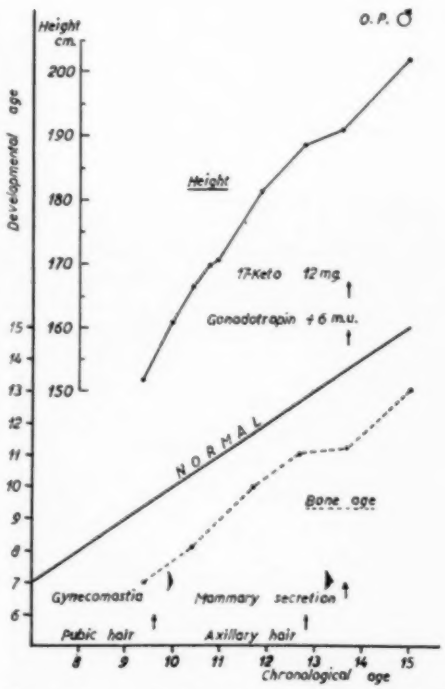



**Fig. 1.** Growth and osseous and sexual development of O. P. Pubic hair and mild gynecomastia appeared as a result of testosterone treatment. A temporary decrease in the rate of growth occurred at the age of 10 years, 6 months following roentgen therapy.

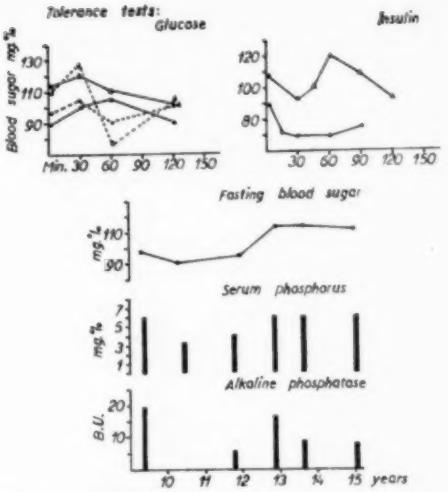



**Fig. 2.** Biochemical determinations at various times from 9 to 15 years of age for O. P. References: .——.——., 9 years 9 months; ----------, 11 years 10 months; Δ——Δ——Δ, 12 years 10 months; and Δ----Δ----Δ, 15 years. Roentgenotherapy at the age of 10 years 5 months.

The skin was lax and thick, forming wide folds, especially noticeable in the neck and hands. The muscles were smooth and apparently of normal strength. External genitalia showed prepuberal characteristics, and there was no pubic or axillary hair. Reflexes and fundi oculi were normal. The visual fields showed a concentric restriction predominantly on the temporal side and more marked in the right eye.

**Laboratory findings.** Roentgenograms showed an enlarged sella turcica and a bone age of 8 years. Blood count and urinalysis were normal. Serum revealed the following: urea, 30 mg. per 100 ml.; cholesterol, 264 mg. per 100 ml.; phosphorus, 6 mg. per 100 ml.; alkaline phosphatase, 20 B.U.; fasting glucose, 99 mg. per 100 ml., and BMR, 12 per cent.

**Clinical course.** Growth and osseous and sexual development, as well as the pattern

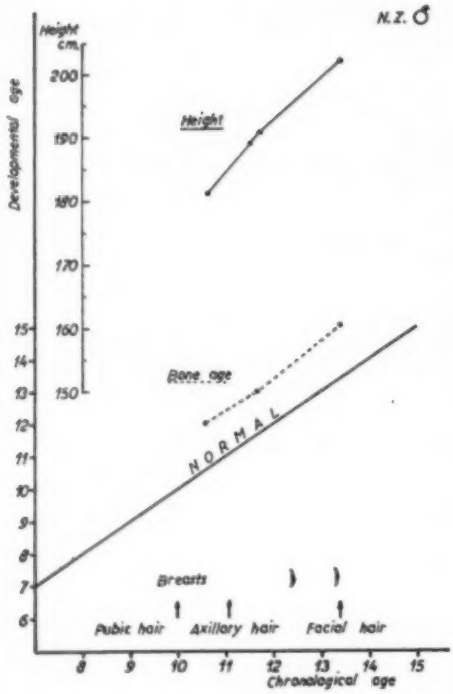


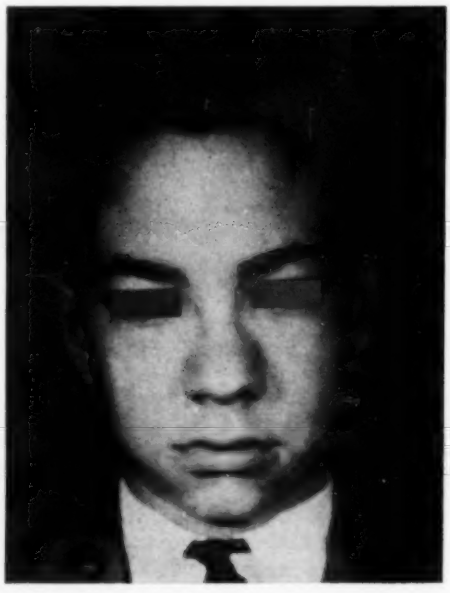

Fig. 3. Growth data of N. Z. (Case 1).

of some biochemical findings, may be followed in the respective charts (Figs. 1 and 2).

When the boy was 9 years 6 months old, treatment was started with 10 mg. of testosterone propionate every other day for 2 months. He then showed growth of pubic hair, mild gynecomastia, and lessening intensity of headaches. Roentgen therapy was started at 10 years 5 months of age and a surface dose of 6,000r was given with the following results: disappearance of the headaches, lowering of serum phosphorus, and temporary slowing of growth. No substantial change was observed in the visual fields.

At the age of 13, an increase of pubic hair and growth of axillary hair began. Gynecomastia was more marked and a milky secretion could be obtained from the nipples. A urinary gonadotropin determination was positive at 6 m. u. Dorsal kyphosis became apparent and the disproportionate enlargement of the extremities continued to be a remarkable feature. These will be compared with that of other overgrown patients.

4A.



4B.

Figs. 4*A* and 4*B*. O. P., age, 9 years 4 months; height, 60¼ inches; and bone age, 6 to 7 years.

## CASES OF OVERGROWN NONACROMEGALIC PATIENTS

**Case 1.** N. Z., a boy of Russian extraction, weight 4,700 grams at birth. His parents were tall and the height of some relatives reached 79 inches. The boy had always been the tallest among children his age.

He was seen for the first time when he was 10 years 7 months old. His height was 71¾ inches, the upper/lower ratio 0.96, and he weighed 75 kilograms. There was puberal development with a moderate amount of pubic hair but no axillary hair. The neurological examination showed no abnormal signs. Fundi oculi, visual fields, and sella turcica were normal. Bone age was 12 years. A glucose tolerance test revealed fasting 98 mg. per 100 ml.; 30 minutes, 143 mg.; 60 minutes, 105 mg.; and 90 minutes, 103 mg. Serum phosphorus was 5.6 mg. per 100 ml.

When the patient was 13 years 5 months old, his height reached 80 inches, but no acromegalic features were present. The growth chart (Fig. 3) is shown in comparison with the case reported above.

**Case 2.** S. P. was a boy of Italian origin. His parents were tall; in the mother's family there was an average height of 75 inches.

He was first seen when he was 14 years 2 months old. His height was 74½ inches, the upper/lower ratio 0.86, and he weighed 78 kilograms. He had always been tall for his age and recently had been growing more rapidly. Growth of pubic and axillary hair had been present for 1½ years. External genitalia showed pubertal development and there was a moderate gynecomastia already in involution. There were no neurological signs of disease. Fundi oculi, visual fields, and sella turcica were normal. Bone age was 13 years. Glucose tolerance test indicated fasting, 96 mg. per 100 ml.; 30 minutes, 110 mg.; 60 minutes, 102 mg.; and 90 minutes, 110 mg. Insulin tolerance test revealed fasting, 98 mg. per 100 ml.; 30 minutes, 86 mg.; 60 minutes, 92 mg.; 90 minutes, 98 mg.; 120 minutes, 98 mg.; and 150 minutes, 96 mg. Calcium was 11.8 mg. per 100 ml.; phosphorus, 4.6 mg. per 100 ml.; and alkaline

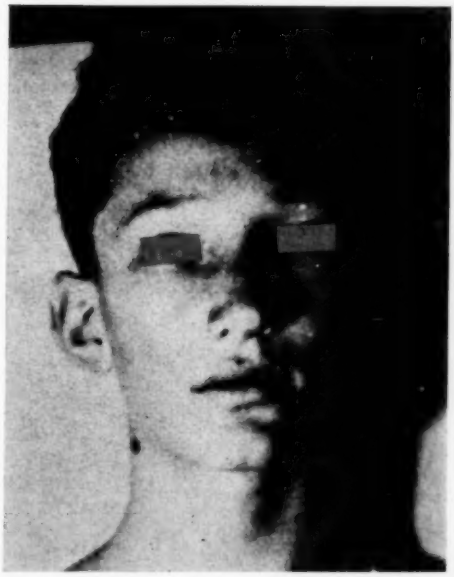


**Fig. 5.** N. Z. (Case 1), age 10 years 7 months; height, 71¾ inches; and bone age, 12 years.

**Fig. 6.** O. P., age, 13 years 7 months; height, 75 inches; and bone age, 11 years.

**Fig. 7.** S. P. (Case 2), age, 14 years 2 months; height, 74½ inches; and bone age, 13 years.

phosphatase, 19 B.U.

At the age of 15 years 8 months he was 76½ inches tall and there were no acromegalic signs.

**Case 3.** M. R., a girl, weighed 3,700 grams at birth. Her father's height was 73¾ inches and some relatives were even taller. She had always been tall for her age.

When first seen at the age of 7 years 11 months her height was 58¼ inches, corresponding to an age of 12 years 6 months, the upper/lower ratio 1.03, and she weighed 48 kilograms. Physical examination revealed no puberal development and no neurological signs of disease. Fundi oculi, visual fields, and sella turcica were normal. Bone age was 11 years. A glucose tolerance test revealed fasting, 104 mg. per 100 ml.; 30 minutes, 135 mg.; 60 minutes, 131 mg.; 90 minutes, 96 mg.; and 120 minutes, 111 mg. Serum indicates calcium, 11.6 mg. per 100 ml.; phosphorus, 5.6 mg. per 100 ml.; and alkaline phosphatase, 12 B.U. Urinary gonadotropin was negative for 6 m. u. and neutral 17-ketosteroids were 5 mg. per 24 hours.

At the age of 8 years 7 months her height was 60 inches and there were no acromegalic features in the patient.

The only outstanding features in all 3 cases are the rapid growth and family history of tall persons. None of these cases show neurological or roentgenological signs which would lead to the suspicion of a pituitary adenoma. On this basis, a diagnosis of constitutional overgrowth was made, after excluding hyperthyroidism, eunuchoidism, sexual precocity, and arachnodactyly.

## ANALYSIS OF THE ACROMEGALIC FEATURES

Appearance, roentgenograms, and comparison of the shoe size are taken into consideration in analyzing the acromegalic changes.

**Head.** The face of the patient when first seen is shown in Fig. 4. The features are coarse. Ears, nose, and lips are thick. The soft tissues are thickened as can be noticed in the upper eyelid and in the folds of the skin of the neck. There was no prognathism at that time, but later it became apparent (Fig. 6). The faces of 2 (Cases 1 and 2) in Figs. 5 and 7 do not manifest the above described features.

At 13 years of age mastication became difficult because of malocclusion. The mandible was elongated and the interdental spaces were increased (Fig. 8).

The lateral view of the skull shows the enlarged sella turcica and the marked pneumatization in comparison with another (Case 2) (Fig. 9).

**Upper extremities.** The aspect of the hands is shown in Fig. 10. However, the comparative study of the x-rays of the hands is more demonstrative of the acromegalic characteristics. The soft tissues appear thickened and the bones widened, particularly in the phalanges which are wider at the distal end. This is especially noticeable in the third phalanx. The cortex is extended and the overexpanded cancellous bone shows gross trabeculae. Although the length of the hand slightly exceeds that of the

comparative roentgenograms, its width makes it appear shorter (Figs. 11 and 12).

**Lower extremities.** The same characteristics of the hands appear in the feet. The disproportionate enlargement is followed by the frequent change of shoe size.

When he was 9 years 4 months old and 60¼ inches tall he wore shoes size 37 (Argentine size) and at age 15 and a height of 80 inches he needed a shoe size 54. N. Z. (Case 1), 13 years 5 months old and 80 inches tall, wore shoes size 48 and S. P. (Case 2) at age 14 years 2 months and a height of 74½ inches wore shoes size 45. The latter 2 shoe sizes are considered normal for those heights.

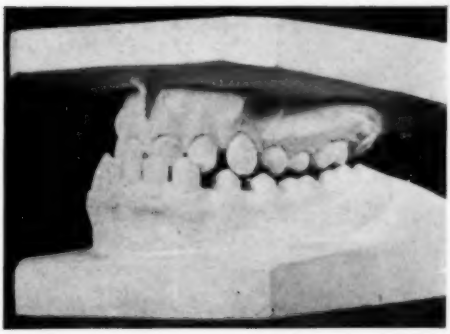
## DISCUSSION

Several cases of acromegaly before adulthood have been described in the literature. Atkinson[4] reviewed 32 cases, some of them doubtful ones. Five well-documented cases from a personal review[5-18] are summarized in the addendum.

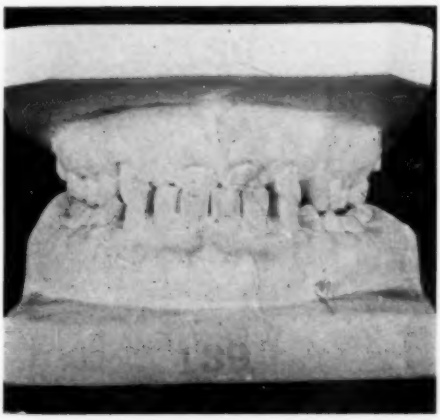
In these, as well as in the present case report, the clinical manifestations of onset and course are similar to acromegaly in the adult. Rapid growth makes the only difference, since it does not occur in adults but is added to symptomatology in children.

Here, too, acromegaly is as Marie[1] stated an "initial phenomenon." It is progressive, but is concomitant with, but not secondary to, the longitudinal growth, which suggests that both manifestations are perhaps of the same cause. The idea of an abnormally high secretion of growth hormone would logically explain the pathologic mechanism, affecting both the endochondral and the periosteal bone formation, therefore lengthening and widening the bones without hastening fusion as well as thickening the skin and the subcutaneous tissue.

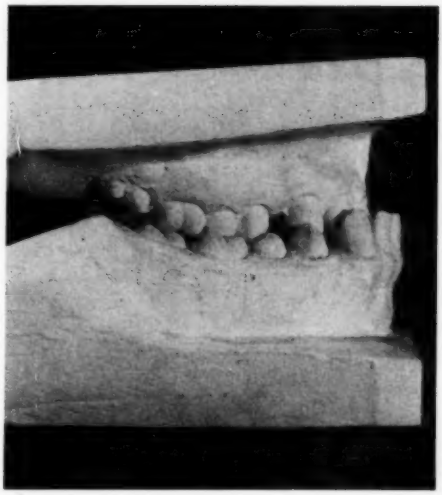
On the contrary, no acromegalic changes are found in the cases of overgrowth of the constitutional type, even when the epiphyses are fused as in the typical case of Gigon,[19] a 22-year-old female 81½ inches tall. Thus there seems to be a clear clinical difference in these 2 types of excessive growth. The



8A.



8B.



8C.

**Figs. 8A, 8B, and 8C. O. P., age 13 years. Frontal and lateral views of buccal cast show prognathism and increased interdental spaces. (Made by Dr. Ramon Torres.)**

quantitative determination of growth hormone in each type of patient surely will be of great value in establishing the relationship between acromegalogigantism and the so-called constitutional gigantism.

## SUMMARY

A case of acromegaly and gigantism in a child with clinical evidence of pituitary adenoma is reported.

The acromegalic features appearing at the age of 9 years 4 months are studied in comparison with those of 3 constitutionally overgrown patients who showed neither thickening of the soft tissue nor roentgenologic changes of the bones which are characteristic of acromegaly.

In the present case (acromegalogigantism), as in other cases reviewed, acromegaly occurs as an early manifestation simultaneously with increased growth, suggesting that both manifestations depend on the same etiologic factor rather than on the maturity of bones.

9*A*.

9*B*.

**Figs. 9*A*** and **9*B***. Lateral view of the the skull. *9A*, O. P., 13 years 7 months old; *9B*, S. P. (Case 2), 14 years 2 months old. (Same photographic reduction of the original roentgenograms.)

**Fig. 10.** O. P.'s hands when he was 9 years 4 months old.

Our thanks to Dr. Lawson Wilkins who encouraged me to write this report some time ago and in whose honor it is now published, to Dr. and Mrs. Jorge M. Martin for helping in the English translation, and to Dr. Ernesto Ferretti for his faithful collaboration with the photographs.

## ADDENDUM

**Surmont's[5] case.** This 14-year-old girl was of short stature; sexual development was normal. Shortly after puberty she complained of amenorrhea, headaches, nausea, and loss of vision. At that time her growth became more rapid and acromegalic signs became apparent as can be seen in the picture in the original paper. At 18 years 6 months her height was 66¼ inches. No x-rays were reported.

**Schultze and Fisher's[8] case.** Vomiting, headaches, and dizziness began when this girl was 11 years old; rapid growth and increasing size of the hands and feet were also noted at that time. At the age of 14 years her height was 66 inches. There was a large mandible, protrusion of the inferior teeth, and prominence of the occiput. The nose was wide and thick and the tongue

11A.

11B.

**Figs. 11*A* and 11*B*.** X-rays of the hand. **11*A*,** O. P., 9 years 4 months; height, 60¼ inches; **11*B*,** M. R. (Case 3), 8 years 7 months; height, 60¼ inches.

was enlarged. Fundi oculi revealed papilledema and optic atrophy in both eyes. There was decreased hearing. No glycosuria was present. She died at 15 years of age. At necropsy there was a pituitary adenoma. The bones were thick and the spongy bone was overexpanded. The skin, both epidermis and corium, was thick. Photographs and radiographs are shown in the paper.

**Verse's[10] case.** Headaches, loss of vision, and typical signs of acromegaly started in this boy at the age of 14. The hands and feet were large and coarse. The nose was wide and thick; the mandible was enlarged; macroglossia was present. There was an enlarged sella turcica and marked pneumatization of the skull. At necropsy a pituitary tumor of eosinophilic type of cells protruding into the left cavernous sinus was noted. No pictures or radiographs are shown.

**Rodriguez's[11] case.** Rapid growth and sporadic headaches were noted in this girl at 4 to 5 years of age. At the age of 7 or 8 years her rapid growth was more marked and disproportionate (acromegalic tendency). There were visual disturbances. At 11 her height was 69¼ inches and her face was coarse. The sella turcica was large and the visual fields showed bitemporal hemianopsia. At the age of 16 she reached a height of 79½ inches. No radiographs are shown in this paper.

**Catola's[12] case.** Rapid growth and enlargement of the hands, feet, and face began at the age of 14 years in this boy. When he was 18 years old his height was 71 inches. Two years later he complained of intense headaches, sleepiness, sexual frigidity, and mental impairment. At the age of 25 years he was 76½ inches tall. He had scarce pubic hair, scoliosis, enlarged sella turcica, and no epiphyseal fusion in the x-rays of the hands. The author demonstrates in this paper the existence of acromegaly prior to the closure of the epiphyses.

## REFERENCES

1. Marie, P.: Hypertrophie singuliére non congenitale des extrémities supérieures, in-

12A.

12B

**Figs. 12*A* and 12*B*.** X-rays of the hand. **12*A*,** O. P., 13 years 7 months; height, 75 inches; **12*B*,** S. P. (Case 2), 14 years 2 months; height, 74½ inches. (The roentgenograms are proportionally reduced from the originals.)


férieures et céphalique, Rev. de méd., Paris **6:** 297, 1886.

2. Arnold, J.: Acromegalie, Pachyacrie oder Ostitis? Beitr. path. Anat. **10:** 1, 1891.
3. Brissaud, E., and Meigge, H.: Gigantisme et acromégalie, J. de méd. et chir. prat. **66:** 49, 1895.
4. Atkinson, F. R. B.: The Onset of Acromegaly Before 15 Years of Age, Brit. J. Child. Dis. **28:** 121, 1931.
5. Surmont, H.: Acromégalie à début précoce, Nouv. Icon. de la Salp. **3:** 147, 1890.
6. Costa, J. P.: Un caso de acromegalia, Rev. Soc. méd. argent. **2:** 384, 1894.
7. Viton, J. J.: A propósito de un caso de acromegalia, Rev. Soc. méd. argent. **15:** 200, 1907.
8. Schultze, F., and Fisher, B.: Zur Lehre von der Akromegalie und Ostearthropathie hypertrophiante, Mitt. Grenzgeb. Med. u. Chir. **24:** 607, 1912.
9. Salle, V.: Über einen Fall von angeborener abnormer Größe der Extremitaten mit einen an Akromegalie erinnernden Symptomenkomplex, Jb. Kinderhilk. **75:** 540, 1912.
10. Verse: Zwei Falle von Akromegalie mit Hypophysentumoren, München. med. Wchnschr. **62:** 269, 1915.
11. Couce, F., and Poyales, F.: Un caso de gigantismo acromegálico, Pediat. Española **8:** 271, 1919.
12. Catola, M. G.: Un cas d'acromégalo-gigantisme. Quelques remarques symptomatologiques radiologiques et pathogéniques, Rev. neurol. **29:** 712, 1922.
13. Rodriguez, B.: Un cas de gigantisme acromégalique (Étude clinique), Rev. neurol. **29:** 716, 1922.
14. Nobecourt: Sur une fille atteinte d'hypertrophie staturales et d'acromégalie, Rev. gén. de clin. et de thérap. **39:** 482, 1925.
15. Stolkind, E.: Case of Acromegaly in a Girl Aged 16 Years, With Congenital Heart Disease (Aortic Stenosis), Proc. Roy. Soc. Med. **16:** 22, 1922 to 1923.
16. Conti, G.: Un caso singolare di acromegalia infantile, Endocrinol. e Pat. Costit. **6:** 5[illegible], 1931.
17. Gareiso, A., Diehl, D., and Schere, S.: Contribucion al estudio de la acromegalia precoz, Arch. argent. de Pediat. **5:** 157, 1934.
18. Simpson, S. L.: Acromegaly and Gigantism, Post Grad. M. J. **26:** 201, 1950.
19. Gigon, A.: Über Riesenwuchs und Zwergwuchs, Schweiz. Arch. Neurol. u. Psychiat. **8:** 153, 1921, and in Schinz, R., Baensch, W., Friedl, E. and Uehlinger, E., editors: Roentgendiagnóstico, vol. 2, ed. 5, Barcelona, 1954, Salvat Editores, p. 1114.

# Studies on thyroid hormone metabolism in children

Heskel M. Haddad, M.D.*

BETHESDA, MD.

THE metabolism of the thyroid hormones in children is not adequately understood. The theory that the child may have higher needs for thyroid hormones than the adult was based on studies with desiccated thyroid and thyroxine in the treatment of hypothyroid children and infants.[1-3] However, estimations of serum protein-bound iodine (PBI)[4-6] and of butanol-extractable iodine (BEI)[5, 7, 8, 9] showed that infants and children over 1 week of age maintained a circulating level of thyroid hormone comparable to that of adults. During the first week of life, PBI and BEI values are significantly higher than they are in adults[4, 5, 8, 9] These high values are related to an increased serum thyroxine-binding capacity, rather than to hyperthyroidism.[10]

Thyroidal $I^{131}$ uptake in children has been studied by several investigators.[6, 11, 22] The results obtained from different laboratories show a wide variation. When some of these $I^{131}$ studies in children are compared with the published studies on adults, an impression is created that euthyroid children have a "hyperactive" state of thyroid function.[14, 19, 20, 22] If the thyroid of normal children is indeed hyperactive, it follows that children must maintain a higher peripheral utilization of thyroid hormone than adults. It is the purpose of this paper to re-examine this aspect of thyroid function in children. Since thyroxine and triiodothyronine constitute the primary physiologically active hormones of the thyroid,[23] the peripheral metabolism of exogenously administered $I^{131}$-labeled thyroxine and triiodothyronine was studied in children. Studies on serum thyroxine-binding protein are presented as well.

*From the National Institute of Arthritis and Metabolic Diseases and the National Heart Institute, National Institutes of Health, Bethesda, Md.*

*Part of this study was performed while the author was a U. S. Public Health fellow, the National Institute of Arthritis and Metabolic Diseases.*

*Present address: Washington University School of Medicine, Department of Ophthalmology, 640 S. Kingshighway, St. Louis 10, Mo.*

## MATERIALS AND METHODS

**Clinical material.** Nineteen children, aged 3 to 9 years, were included in the studies. All were institutionalized because of mental retardation.* They were all euthyroid clinically and showed no evidence of endocrine or metabolic disturbance. Their growth and physical and osseous development were normal for their chronologic ages. Seventeen children were given $I^{131}$-labeled L-thyrox-

*Selected from the District of Columbia Children's Training School, Laurel, Md.

ine*; serum thyroxine binding capacity was assayed in 6 of them. Five children were given $I^{131}$-labeled L-triiodothyronine.*

**Thyroxine-binding capacity.** The techniques employed in this study have, for the most part, been described in detail by Robbins.[24] Reverse-flow electrophoresis in horizontally suspended filter paper strips was done with barbital buffer at pH 8.6 and ionic strength of 0.1. It differed from the conventional method in the point of application of the protein and in the hydrodynamic balance of the system.

Serum obtained from 6 euthyroid children (aged 3 to 5 years) was added to appropriate quantities of $I^{131}$-labeled L-thyroxine previously dried in vacuo. The mixtures were allowed to equilibrate for at least 5 hours at 4° C. The final concentration of thyroxine in these mixtures was assessed by iodine analysis of the serum and of the original thyroxine solution and by radioactivity recovery analyses to correct for losses on glassware or during evaporation. The serum protein-bound iodine (PBI) was assumed to be entirely thyroxine, for the purposes of the calculations.

Iodine analyses† were performed by a modification of the Zak procedure.[25] The serum PBI was measured by the Barker alkaline-ash method.[26-27]

Radioactivity on paper strips was measured by a continuously recording counting rate meter and an end-window Geiger-Mueller tube. Relative quantities of radioactivity in different areas on a strip were determined by planimetry of the radioactivity records (Fig. 1).

**Metabolic studies of thyroxine and triiodothyronine.** Tracer doses of $I^{131}$-labeled L-thyroxine or L-triiodothyronine were administered to 19 euthyroid children, whose thyroid $I^{131}$ uptake was suppressed with methimazole (Tapazole) 1 day before and

*Both radioactive thyroxine and triiodothyronine were obtained from Abbott Laboratories, Oak Ridge, Tenn. They were tested for radiochemical purity by paper chromatography, and were purified, when necessary, to a purity of 90 per cent or better.

†Performed by J. Benotti, Boston Medical Laboratories, Boston, Mass.

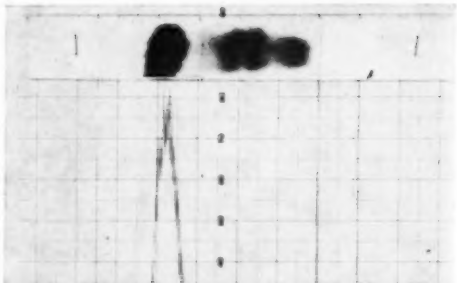



**Fig. 1.** Reverse-flow zone electrophoresis of labeled L-thyroxine-serum mixture. The paper strip, stained for protein, is aligned with the record of its radioactivity content.

throughout the study. Plasma samples were collected at 10 and 30 minutes, at 1, 2, 4, 6, 8, 12, and 24 hours, and at daily intervals thereafter for 10 days in the case of thyroxine, and for 5 days in the case of triiodothyronine. The radioactivity of the plasma, as well as that of the stool and urine, was counted in a well-type scintillation counter. The results are expressed as a fraction of the dose per liter of plasma or per total urine or stool collection.

Plasma disappearance curves were plotted for each case against time on semilogarithmic coordinates (Figs. 2 and 3). The curves could be reduced to a minimum of 2 exponential components: an initial fast component followed within 6 to 8 hours by a slow component, whose half time was considered as the biologic half-life ($T\frac{1}{2}$) for the labeled compound.

Calculation of the data was based on a 2-compartment system model,[28] and was estimated according to mathematical calculations reported elsewhere.[29]

## RESULTS

**Serum protein-bound iodine (PBI).** Thirty-three estimations of serum PBI were

made on the 19 euthyroid children included in these studies. Serum PBI ranged between 4.4 and 8.0 μg per 100 ml. (Table I), with a mean of 6.09.

**Thyroxine-binding capacity.** Twelve assays of serum thyroxine-binding protein were made in 6 euthyroid children. Qualitatively, no difference was detected between thyroxine-protein interaction and that of normal adults. At concentrations of the order of 0.2 μg of added thyroxine per milliliter of serum, most of the serum iodide had an electrophoretic mobility, at pH 8.6, intermediate between alpha-1 and alpha-2 globulin (Fig. 1). The remainder was associated with albumin (5 per cent or less was sometimes found in the pre-albumin zone). Quantitatively, the results of the thyroxine-binding capacity of the thyroxine binding protein ranged between 0.19 and 0.32 μg of thyroxine per 1 ml. of serum, with a mean of 0.25 ∓ S.E. 0.011 (Table I). In 14 adults, whose thyroxine-binding protein was estimated by Robbins,[33] the thyroxine-binding capacity ranged between 0.16 and 0.34 μg of thyroxine per 1 ml. of serum, with a mean of 0.22 ∓ S.E. 0.015.

**Thyroid hormone metabolism.** In Table I the data obtained on radioactive thyroxine and triiodothyronine turnover rates in children are presented and compared with data obtained in adults by other workers. It is evident from Figs. 2 and 3 that the rate of disappearance for triiodothyronine is much faster than that of thyroxine. Whereas the mean half-life ($T\frac{1}{2}$) for thyroxine was 4.95 days, that for triiodothyronine was 1.13 days, indicating a faster rate of metabolism for the latter. This was corroborated when the fractional turnover rates as estimated from the 2-compartment model system for the 2 hormones were determined. Thyroxine had a fractional turnover rate of 13.9 per cent of the dose per day, while that for triiodothyronine was 72.2 per cent of the dose per day. The extrathyroidal endogenous triiodothyronine pool in micrograms and its rate of degradation in micrograms per day could not be estimated, because the level of endogenous triiodothyronine in the serum is not known. The values for thyroxine pool in micrograms and its rate of degradation in micrograms per day were estimated on the assumption that the serum PBI value was 100 per cent thyroxine. The total volume of distribution for thyroxine (2.406 liters) did not differ significantly from that for triiodothyronine (2.086 liters).

## DISCUSSION

The data of the studies, as summarized in Table I, indicate that the circulating thyroid hormone is maintained at the level of an adult in the child, without a significantly different thyroxine-binding capacity. Children, however, maintain their euthyroid status at the expense of an increased rate of utilization (as expressed in the fractional turnover rate) of thyroid hormone.

In Table II, data on thyroid function at various periods of childhood, obtained by several investigators, are presented. From the PBI and BEI values, it is evident that only during the first week of life is there any significant rise in the child's PBI and BEI compared to their levels in the adult.[4-9] Initially this led to the belief that the thyroid of the newborn infant was hyperactive during the neonatal period.[4, 5] However, $I^{131}$ uptake studies in premature and full-term infants failed to corroborate this.[18] Later, Robbins and Nelson[10] showed that this elevation of serum PBI and BEI was merely a function of an increased thyroxine-binding protein in the serum of the neonate.

Since the advent of $I^{131}$ for the study of thyroid function, several reports on $I^{131}$ uptake by the thyroid have been made in euthyroid children.[6, 11-22] However, as is evident from Table II, the data show considerable variation. Many difficulties are encountered in the study of the child's thyroid $I^{131}$ uptake: (a) Small quantities of the radioactive solution, when given orally, may be lost by regurgitation or by drooling. (b) Contamination of the diaper with the $I^{131}$ in the urine could increase the background. (c) Estimates of the size of the thyroid gland and the contribution of the extrathyroidal neck counts may introduce certain errors,

**Table I.** Summary of data of thyroid hormone metabolism in 19 euthyroid children as compared with reported values in adults

| | Range | Child data | | Adult means |
|---|---|---|---|---|
| | | Mean | ∓ S.E. | |
| PBI (μg per cent) | 4.4 - 8.0 | 6.09 | 0.16 | 6.1[27, 32] |
| Thyroxine-binding protein thyroxine (μg/ml.) | .19 - .32 | .25 | 0.011 | .22[33] |
| *Thyroxine rates* | | | | |
| Biologic half-life (days) | 3.9 - 5.9 | 4.95 | 0.13 | 6.7[30, 32] 6.6[31] |
| Total volume of distribution (liters) | 1.488- 3.964 | 2.406 | 0.156 | 9.4[31] |
| Extrathyroidal thyroxine (μg) | 101.6 -195.4 | 145.2 | 7.7 | 548[32] 508[31] |
| Turnover (per cent/day) | 10.5 - 18.7 | 13.9 | 0.47 | 10.5[32] 10.56[31] |
| Degradation (μg/day) | 12.5 - 27.8 | 19.7 | 1.04 | 57[32] 53.6[31] |
| *Triiodothyronine rates* | | | | |
| Biologic half-life (days) | 0.98 - 1.33 | 1.13 | 0.05 | 2.6[30] |
| Total volume of distribution (liters) | 1.723- 2.460 | 2.086 | 0.182 | |
| Turnover (per cent/day) | 62.5 - 79.3 | 72.24 | 4.08 | 27.4[30] |

**Table II.** Reported studies on thyroid function in children in chronologic order ($I^{131}$ uptake is expressed as per cent of the administered dose unless stated otherwise. The corresponding values for the various age groups are listed in the same succession as the group. All values given are those of the means except when only a range of the values was available.)

| *Author and reference No.* | *Year* | *Study* | *Age range* | *Results* |
|---|---|---|---|---|
| Quimby and McCune[11] | 1947 | $I^{131}$ uptake | < 1, 1-4, 5-14 years | 12.3, 9.9, 13.5 (per cent/24 hours) |
| Lowry et al.[12] | 1949 | $I^{131}$ uptake | ½-14 years | 400-800 (% of 10 minute count/24 hours) |
| Danowsky et al.[4] | 1951 | PBI | 7, 8-39 days | 10.3, 6.8 (μg per cent) |
| Reilly and Bayer[13] | 1952 | $I^{131}$ uptake | 9/12-15 years | 16 (%/24-96 hours) |
| Man et al.[5] | 1952 | PBI | < 7, > 30 days | 8.7, 6.5 (μg %) |
| | | BEI | | 7.7, 5.8 (μg %) |
| Pickering and Miller[6] | 1953 | PBI | 1-10 years | 5.0 (μg %) |
| | | $I^{131}$ uptake | | 12-23 (%/24 hours) |
| Van Middlesworth[14] | 1954 | $I^{131}$ uptake | < 7 days | 69.7 (%/24 hours) |
| Durham et al.[7] | 1954 | BEI | 2/12-12 years | 4.5-7.3 (μg %) |
| Jaimet and Thode[15] | 1955 | $I^{131}$ uptake | ? | 31 (%/24 hours) |
| | | $PBI^{131}$ | | 29 (%/24 hours) |
| Friedman[16] | 1955 | $I^{131}$ uptake | ½-16 years | 21.4 (%/24 hours) |
| Reilly et al.[34] | 1955 | $PBI^{131}$ | ? | 6.1 and 17.0 (counts per second %/24 and 48 hours) |
| Talbot[17] | 1955 | $I^{131}$ uptake | < 2 years | 12 (%/24 hours) |
| Martmer et al.[18] | 1956 | $I^{131}$ uptake | Premature or full-term | 29.8, 33.4 (%/24 hours) |
| Gali et al.[19] | 1956 | $I^{131}$ uptake | 4-11 years | 43.5 (%/24 hours) |
| Tubiana and Ravaud[20] | 1956 | $I^{131}$ uptake | < 2, 2-6, 7-11 years | 10, 42, 40 (%/24 hours) |
| Sheline et al.[21] | 1957 | $I^{131}$ uptake | <18 years | 22.1 (%/24 hours) |
| Oliner et al.[22] | 1957 | $I^{131}$ uptake | 1-4, 5-9 years | 31.5, 27.6 (%/24 hours) |
| | | $PBI^{131}$ | | 0.125, 0.049 (%/liter/24 hours |
| Pickering et al.[8] | 1958 | BEI | 5, 20, 39 days | 9.9, 5.7, 6.5 (μg %) |
| Man, Shaver, and Cooke[9] | 1958 | BEI | 2-5 days | 7.9 (μg %) |

especially if the uptake value is to be compared with that of an adult. (d) The interpretation of the $I^{131}$ uptake, as expressed in per cent of the dose, is misleading, since due allowance is not made (comparing the child with the adult) for possible differences in iodide pools, rate of disappearance of $I^{131}$ from the serum and of $I^{131}$ excretion in the

urine, all of which could influence the $I^{131}$ uptake by the thyroid. Thus, whereas $I^{131}$ uptake study may be rewarding as a diagnostic tool in thyroid disease of the child, its definitive value in the study of the normal physiologic function of the child's thyroid is uncertain. Similar limitations hold true for the study of the $PBI^{131}$-conversion ratio.

The data for thyroxine and triiodothyronine metabolic rates, as presented in Table I, were estimated by a method different from that used for the adult data reported by Sterling and associates[30, 32] and Ingbar and Freinkel.[31] When calculated by the intercept rather than the 2-compartment system method, the data for children become about 5 to 10 per cent higher. Furthermore, when thyroxine extrathyroidal pool and degradation rate in micrograms per day are expressed per unit of surface area or of body weight, the disparity between the data for child and adult almost disappears. Actually, the degradation rate becomes somewhat higher in the child than in the adult: child's mean = 32.0 μg per square meter per day or 1.29 μg per kilogram per day and adult mean = 29.4 μg per square meter per day or 0.75 μg per kilogram per day.[32]

Unfortunately, we have no knowledge as to the actual level of thyroxine and triiodothyronine in the circulation. For this reason, the complete interpretation of the triiodothyronine data is not possible. Superficially it appears that the euthyroid child utilizes his peripheral triiodothyronine at about 3 times the rate of the adult. This may be indicative of a higher peripheral tissue demand for triiodothyronine, but it may, on the other hand, suggest a normally lower level of triiodothyronine in the child's circulation.

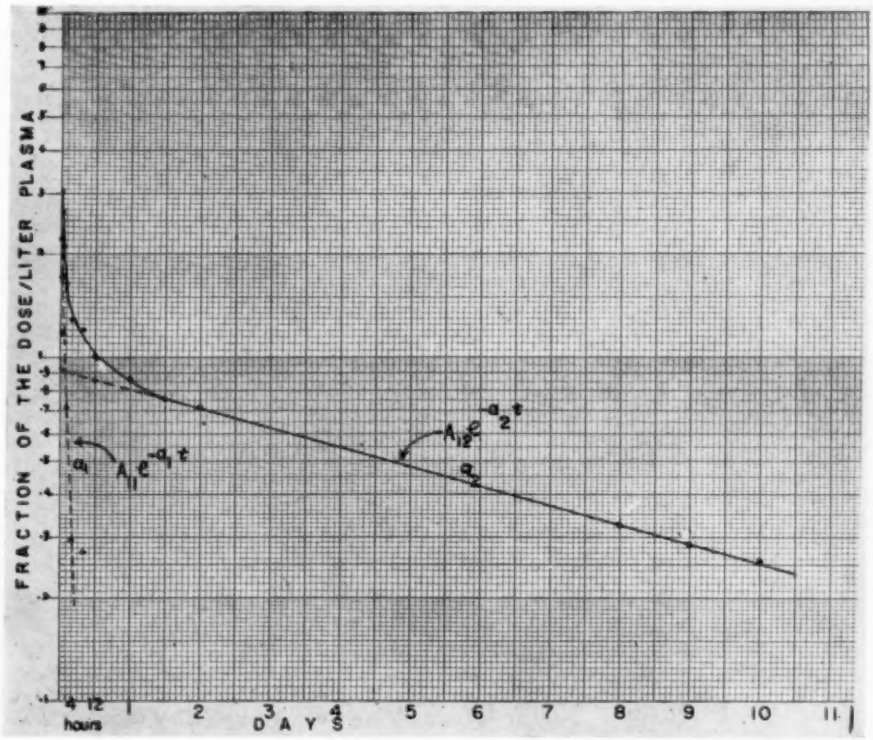



**Fig. 2.** Semilogarithmic plot of the plasma diappearance curve for labeled L-thyroxine.

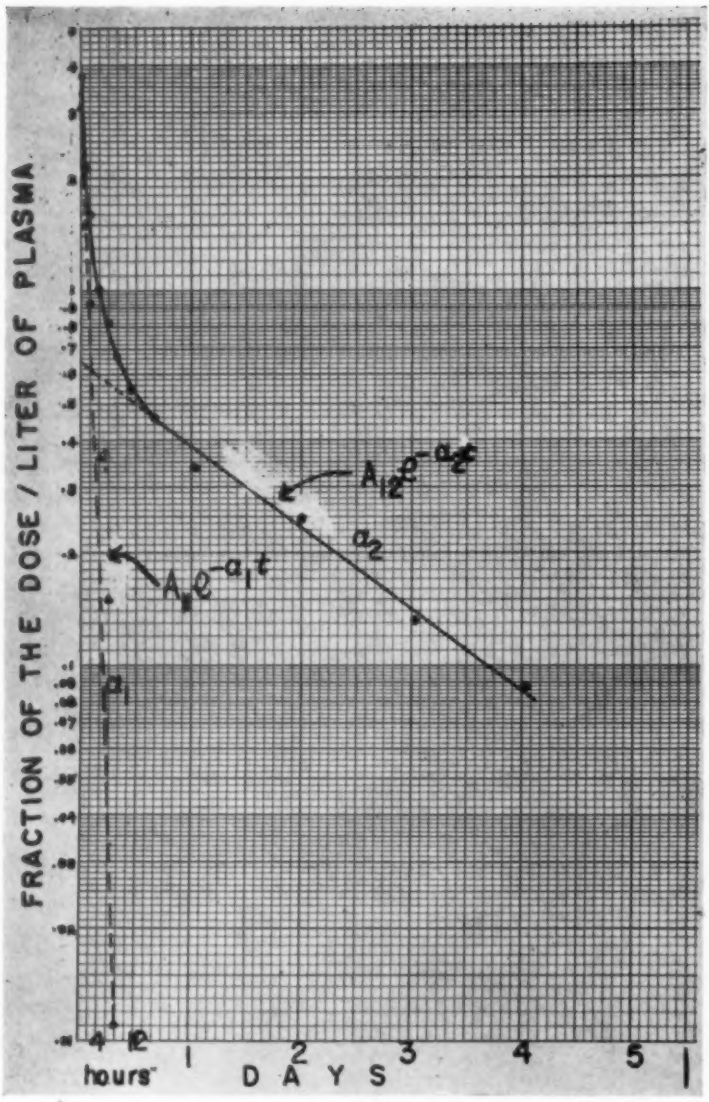



**Fig. 3.** Semilogarithmic plot of the plasma disappearance curve for labeled L-triiodothyronine.

Similarly, the increased turnover rate of thyroxine is explainable on the basis of reduced effective extrathyroidal thyroxine pool in the child.

Thyroxine degradation rates, especially when expressed in micrograms per unit of surface area or body weight, point to a definite increase in peripheral tissue's demand for thyroid hormones in the child as compared to the adult. Again, not until we have a better understanding of the circulating triiodothyronine can we adequately measure the actual demand by the child's tissues for thyroid hormone.

## SUMMARY

Serum protein bound iodine and thyroxine-binding capacity and the rates of

metabolism of $I^{131}$ labeled thyroxine and triiodothyronine were estimated in 19 euthyroid children. While serum protein bound iodine and thyroxine-binding capacity did not differ from adult values, thyroid hormones were utilized at faster turnover rates in the child. The mean half life for thyroxine was 4.95 days and that for triiodothyronine 1.13 days. The mean turnover rates were 19.7 and 72.2 per cent per day, respectively. When expressed in micrograms per unit of surface area or unit of body weight per day, the degradation rate is higher in the child than in the adult.

## REFERENCES


1. Wilkins, L.: Thyroid Medication During Childhood, J. A. M. A. **114:** 2382, 1940.
2. Wilkins, L.: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence, Springfield, Ill., 1950, Charles C Thomas, Publisher, chap. V.
3. Fisher, D. A., Hammond, G. D., and Pickering, D. E.: The Hypothyroid Infant and Child: Therapy With Sodium L-thyroxine, A. M. A. Am. J. Dis. Child. **90:** 6, 1955.
4. Danowsky, T. S., Johnston, S. Y., Price, W. C., McKelvy, M., Stevenson, S. S., and McClusky, E. R.: Protein-bound Iodine in Infants From Birth to One Year of Age, Pediatrics **7:** 240, 1951.
5. Man, E. B., Pickering, D. E., Walker, J., and Cooke, R. E.: Butanol-extractable Iodine in the Serum of Infants, Pediatrics **9:** 32, 1952.
6. Pickering, D. E., and Miller, E. R.: Thyrotropic Hormone in Infants and Children: Differentiation Between Primary and Hypopituitary Hypothyroidism, A. M. A. Am. J. Dis. Child. **85:** 135, 1953.
7. Durham, J. R., Cooke, R. E., Lancaster, J. W., and Man, E. B.: Serum Butanol-extractable Iodine Values of Children Under Ten Years of Age, A. M. A. Am. J. Dis. Child. **87:** 468, 1954.
8. Pickering, D. E., Kontaxis, N. E., Benson, R. C., and Meechan, R. J.: Thyroid Function in the Perinatal Period, A. M. A. J. Dis. Child. **95:** 616, 1958.
9. Man, E. B., Shaver, B. A., Jr., and Cooke, R. E.: Studies of Children Born to Women With Thyroid Disease, Am. J. Obst. & Gynec. **75:** 728, 1958.
10. Robbins, J., and Nelson, J. H.: Thyroxine-binding by Serum Protein in Pregnancy and in the Newborn, J. Clin. Invest. **37:** 153, 1958.
11. Quimby, E. H., and McCune, D. J.: Uptake of Radioactive Iodine by the Normal and Disordered Thyroid Gland in Children: A Preliminary Report, Radiology **49:** 201, 1957.
12. Lowry, G. H., Beierwalters, W. H., Lampe, I., and Gomberg, H. J.: Radioiodine Uptake Curve in Humans: II. Studies in Children, Pediatrics **4:** 627, 1949.
13. Reilly, W. A., and Bayer, D. I.: The Values of the Measurements of Thyroid Uptake and Urinary Excretion of $I^{131}$ in Assessing Thyroid Function of Normal and Congenitally Hypothyroid Children, Pediatrics **40:** 714, 1952.
14. Van Middlesworth, L.: Radioactive Iodide Uptake of Normal Newborn Infants, A. M. A. Am. J. Dis. Child. **88:** 439, 1954.
15. Jaimet, C. H., and Thode, H. G.: Thyroid Function Studies on Children Receiving Cobalt Therapy, J. A. M. A. **158:** 1353, 1955.
16. Friedman, A.: Radioiodine Uptake in Children With Mongolism, Pediatrics **16:** 55, 1955.
17. Talbot, N. B.: $I^{131}$-uptake in Children, in Werner, S. C., editor: The Thyroid, New York, 1955, Paul B. Hoeber, Inc.
18. Martmer, E. E., Corrigan, K. E., Charbeneau, H. P., and Sosin, A.: A Study of the Uptake of Iodine ($I^{131}$) by the Thyroid of Premature Infants, Pediatrics **17:** 503, 1956.
19. Gali, P., Zara, and Gilbert-Dreyfus: La courbe d'$I^{131}$ des enfants normaux, Ann. endocrinol. **17:** 419, 1956.
20. Tubiana, M., and Ravaud, G.: Résultats de 500 tests de fixation intra thyroidienne de l'iode radioactif chez l'enfant, Ann. endocrinol. **17:** 175, 1956.
21. Sheline, G. E., Koulischer, N., and Pickering, D.: Thyroidal Accumulation of Radioiodine in Children, A. M. A. J. Dis. Child. **93:** 391, 1957.
22. Oliner, L., Kohlenbrener, R. M., Fields, T., and Kunstadter, R. H.: Thyroid Function Studies in Children: Normal Values for Thyroidal $I^{131}$ Uptake and $PBI^{131}$ Levels Up to the Age of 18, J. Clin. Endocrinol. **17:** 61, 1957.
23. Roche, J., and Michel, R.: Nature and Metabolism of Thyroid Hormones, Recent Progress in Hormone Research **12:** 1, 1956.
24. Robbins, J.: Reverse-flow Zone Electrophoresis. A Method for Determining the Thyroxine-binding Capacity of Serum Protein, Arch. Biochem. **63:** 461, 1956.
25. Zak, B., Willard, H. M., Myers, G. B., and Boyle, A. J.: Chloric Acid Method for Determination of Protein-bound Iodine, Analyt. Chem. **24:** 1345, 1952.
26. Barker, S. B.: Determinations of Protein-bound Iodine, J. Biol. Chem. **173:** 715, 1948.
27. Barker, S. B., Humphrey, M. J., and Soley, M. H.: The Clinical Determination of Protein-bound Iodine, J. Clin. Invest. **30:** 55, 1951.
28. Berman, M., and Schoenfeld, R.: Invariants in Experimental Data on Linear Kinetics and the Formulation of Models, J. Appl. Physics **27:** 1361, 1956.
29. Haddad, H. M., and Rall, J. E.: Rates of $I^{131}$-labeled Thyroxine Metabolism in Euthyroid Children, J. Clin. Invest. (in press).

30. Sterling, K., Lashof, J. C., and Man, E. B.: Disappearance From Serum of $I^{131}$-labeled l-thyroxine and l-triiodothyronine in Euthyroid Subjects, J. Clin. Invest. **33:** 1031, 1954.
31. Ingbar, S. H., and Freinkel, N.: Simultaneous Estimation of Rates of Thyroxine Degradation and Thyroid Hormone Synthesis, J. Clin. Invest. **34:** 808, 1955.
32. Sterling, K., and Chodos, R. B.: Radiothyroxine Turnover Studies in Myxedema, Thyrotoxicosis, and Hypermetabolism Without Endocrine Disease, J. Clin. Invest. **35:** 806, 1956.
33. Robbins, J.: Personal communication.
34. Reilly, W. A., Scott, K. G., Winters, R. W., and Helwig, H. L.: Anionic Resin Measurement of Protein-bound $I^{131}$ in Euthyroid Children, A. M. A. Am. J. Dis. Child. **89:** 572, 1955.

# The history and present concepts of autoimmunization in thyroid disease

Robert M. Blizzard, M.D.,* and Robert W. Chandler, B.S., M.Sc.

COLUMBUS, OHIO

A FASCINATING new concept of immunology has recently been applied to the study of the thyroid. This is the concept that an organism has the ability to develop antibodies to its own tissues and subsequently to destroy the structures containing the immunizing antigens. It is likely that this phenomenon of autoimmunization and autodestruction is not only of importance in thyroid disease, but also contributes to the pathologic physiology of other diseases now poorly understood.

Acceptance of autoimmunization and autodestruction as possible mechanisms of disease is based on data obtained from both experimental and clinical observations. Injection of homologous brain tissue into animals results in the production of circulating antibodies to brain tissue and encephalomyelitis,[1, 2, 3] and injection of testicular extracts with Freund's adjuvant into guinea pigs and rats produces aspermatogenesis and circulating antibodies.[4, 5] Similarly, circulating antibodies to thyroid tissue appear after homologous thyroid extracts with adjuvant are injected into rabbits, dogs, and guinea pigs. These antibodies are associated with, or result in, histologic alteration of the thyroid gland.[6, 7, 8]

Demonstration of circulating antibodies to adrenal tissue in some patients with Addison's disease,[9] to sperm in occasional cases of orchitis,[10] and to thyroglobulin or thyroid tissue extract in multiple types of thyroid disease[11-15] suggests that autoimmunization is of more than experimental interest and plays an etiological role in human disease.

The nature of the antigens and circulating antibodies associated with thyroid diseases has been studied and the correlation between these antibodies and the pathologic changes in the thyroid has been investigated. A review of these studies elucidating the role of autoimmunization in thyroid disease will be presented in this paper. In addition, present concepts relating to the cytotoxic role of the various thyroid antibodies will be discussed.

## HISTORY IN RÉSUMÉ

The first report of circulating antibodies in the serums of patients with thyroid disease was that of Papazolu[16] in 1911. Utilizing a complement-fixation technique, he demonstrated that the serums of some patients with thyrotoxicosis reacted with ex-

*From the Department of Pediatrics and the Division of Endocrinology, Ohio State University.*

*This work was supported by Grants A-1917 and A-3470, from the Division of Arthritis and Metabolic Diseases, United States Public Health Service.*

**Address, Division of Pediatric Endocrinology, The Johns Hopkins Hospital, Baltimore 5, Md.*

tracts of thyrotoxic glands to fix complement. In 1923, Hektoen and Schulhof[17, 18] produced precipitating antibodies to thyroid extract in animals by injecting extracts of homologous and heterologous thyroid glands. Little work pertaining to the production of antibodies to thyroid tissue was subsequently reported until 1955 and 1956 when Witebsky, Rose, and Shulman[6, 19-22] published a series of articles describing the production of precipitating, complement-fixing, and agglutinating antibodies to extracts of thyroid tissue in rabbits. These antibodies were organ specific, relatively species specific, and frequently associated with pathologic changes of the thyroid suggesting autodestruction.

Roitt and associates,[23] stimulated by the work of Witebsky and colleagues and by the observation that patients with Hashimoto's thyroiditis (lymphadenoid goiter) often have elevated gamma globulin levels[24] and altered liver function tests,[24, 25] investigated the serums of such patients. Circulating antibodies to thyroid extract were found by the use of a precipitin test. More complete data reported by these authors[26] confirmed their initial impression that precipitating antibodies were seldom associated with diseases other than Hashimoto's thyroiditis and spontaneous myxedema.

Subsequently, Witebsky and co-workers[5] demonstrated the presence of agglutinating antibodies in the serums of a large percentage of patients with chronic thyroiditis. On the basis of these findings and the similar pathologic changes observed in the thyroids of patients with Hashimoto's thyroiditis and in the thyroids of animals immunized with thyroid extracts, Doniach and Hudson[27] postulated that thyroglobulin released from the thyroid follicle could induce antibody formation. The antibodies then could combine with the remaining antigen (i.e., thyroglobulin) in the thyroid and form an antigen-antibody complex; this then would induce an inflammatory process with subsequent release of more antigen, increased antibody production, and eventual autodestruction of the thyroid.

The use of the sensitive agglutinating technique enabled Blizzard and co-authors[28, 29] and Roitt and Doniach[11] to demonstrate thyroid antibodies in the serums of patients with thyroid diseases other than Hashimoto's thyroiditis and spontaneous myxedema. Various authors[11, 13, 15] also identified complement-fixing antibodies in serums from such patients.

It soon became apparent that multiple types of antibodies occurred independently or concurrently in the serums of many persons with thyroid disease. The high incidence of these circulating antibodies in patients with multiple types of thyroid disease necessitated reconsideration of the cytotoxic role of each type of antibody on thyroid tissue. Recently, the identification in the serums of several patients with Hashimoto's thyroiditis of a cytotoxic agent[30] which correlates poorly with any of the presently known antibodies suggests that these antibodies may not be cytotoxic.

## TYPES AND INCIDENCE OF ANTIBODIES

**Precipitin antibodies.** These are antibodies that form a precipitate or flocculation upon combining with an appropriate antigen (Fig. 1). Precipitin antibodies to thyroid extract have been detected in the serums of approximately 30 per cent of animals immunized with homologous thyroid extracts emulsified in Freund's adjuvant.[7] In humans, precipitin antibodies occur in approximately 65 per cent of patients with Hashimoto's thyroiditis, 20 per cent of patients with primary myxedema, but rarely in other types of thyroid disease.[26, 31, 32]

More than one precipitin antibody has been demonstrated repeatedly by both the Ouchterlony agar gel plate technique and by precipitin curves. Roitt and associates[32] have demonstrated 1 antibody that upon combination with its antigen (thyroid extract) is soluble in an excess of antibody and, therefore, does not precipitate until optimum proportions of antigen and antibody are present. A second antibody precipitates despite an excess of antibody. Korngold and co-

workers,[33] by combining ultra centrifugal methods and Ouchterlony techniques, have shown that there is one precipitating antibody with a molecular weight of 160,000 in the 7S fraction of gamma globulin and that there is a second precipitating antibody with a molecular weight of approximately 1,000,000 in the 19S fraction.

The antigen for the precipitin antibodies has been found in extracts of both normal and abnormal thyroid glands[32] and has been identified as thyroglobulin.[26, 32] It has not usually been possible by the precipitin technique to demonstrate cross antigenicity of human thyroglobulin with that of species other than the monkey and chimpanzee.[32] This indicates relative species specificity of the antigen.

**Agglutinating antibodies.** These antibodies are capable of agglutinating thyroglobulin coated, tannic acid treated, type "O" human red blood cells. They were first detected in animals immunized with homologous thyroid extracts suspended in Freund's adjuvant.[22] In 1957, Witebsky and co-workers[6] identified agglutinating antibodies in 12 of 19 patients with chronic thyroiditis. Antibodies demonstrable by this technique, in contrast to the precipitating technique, are present in multiple types of thyroid diseases (Table I). It is thought that the agglutinating and precipitating techniques may measure the same antibody, but the agglutinating technique is more sensitive. Evidence for this has been reported by Owen and Smart,[12] who found that an agglutinating serum titer of 1:40,000 was necessary to obtain a positive precipitin test, and by Roitt and Doniach,[11] who found that a positive precipitin test correlated with a high agglutinating titer. Rose,[34] however, has observed a few serums that gave a strongly positive precipitin test and a weak agglutination reaction. Since the agglutinating antibody is possibly the same as the precipitating antibody, it is not surprising that both have been identified as gamma globulins.[33, 35]

Rose and Witebsky[6] found that the antigen for agglutinating antibodies is thyroglobulin. They were able to stimulate the production of agglutinating antibodies in animals by immunizing with relatively pure thyroglobulin as well as with crude thyroid extract. In addition, they demonstrated that thyroglobulin could replace crude thyroid extract as an antigen in the in vitro agglutination reaction of antigen and antibody.[21, 36] When tested by the agglutinating technique, the thyroglobulins of various species have minimal cross antigenicity with those of heterologous species.[37] This indicates relative species specificity of the antibody and the antigen. Relative organ specificity also was demonstrable as most serums containing thy-

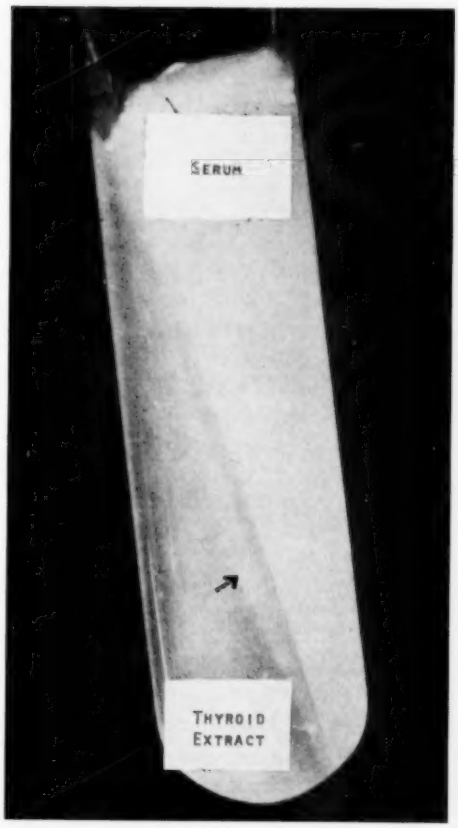



**Fig. 1.** A positive agar gel diffusion test. A line of precipitation is present and marked by the arrow. This is in the interphase between the antigen or thyroid extract and the serum containing the antibodies.

**Table I.** Incidence of agglutinating antibodies to thyroglobulin in thyroid diseases

| | *Total* | + | − |
|---|---|---|---|
| Chronic thyroiditis | 7 | 6 | 1 |
| Acute thyroiditis | 6 | 0 | 6 |
| Subacute thyroiditis | 3 | 1 | 2 |
| Thyrotoxicosis | 103 | 31 | 72 |
| Spontaneous myxedema | 21 | 12 | 9 |
| Nontoxic nodular goiter | 36 | 11 | 25 |
| Diffuse asymptomatic goiter | 9 | 3 | 6 |
| Adolescent goiter | 8 | 0 | 8 |
| Congenital goitrous cretinism | 16 | 2 | 14 |
| Thyroid carcinoma | 3 | 0 | 3 |
| Total | 212 | 66 | 146 |
| Control patients | 219 | 8 | 211 |

**Table II.** Incidence of complement-fixing antibodies to thyroid cellular substance in thyroid diseases

| | *Total* | + | − |
|---|---|---|---|
| Chronic thyroiditis | 5 | 3 | 2 |
| Acute thyroiditis | 5 | 0 | 5 |
| Subacute thyroiditis | 2 | 0 | 2 |
| Thyrotoxicosis | 48 | 11 | 37 |
| Spontaneous myxedema | 12 | 4 | 8 |
| Nontoxic nodular goiter | 26 | 8 | 18 |
| Diffuse asymptomatic goiter | 6 | 1 | 5 |
| Adolescent goiter | 4 | 1 | 3 |
| Congenital goitrous cretinism | 16 | 1 | 15 |
| Thyroid carcinoma | 1 | 0 | 1 |
| Total | 125 | 29 | 96 |
| Control patients | 202 | 1 | 201 |

roid antibodies do not react to a significant extent with extracts of muscle, liver, spleen, or kidney.[8, 28]

**Complement-fixing antibodies to thyroid cellular substance.** As previously noted, antibodies capable of fixing complement were detected in the serums of thyrotoxic patients by Papazolu[16] in 1911. Complement-fixing antibodies also were found by Witebsky and colleagues[19, 22] in rabbits immunized with homologous and heterologous thyroid extracts. It was, however, Trotter and coworkers[38] who, in 1957, first extensively investigated complement-fixing antibodies in patients with thyroid diseases. These authors found complement-fixing antibodies in the serums of patients with thyrotoxicosis, Hashimoto's thyroiditis, and spontaneous myxedema. Roitt and Doniach[11] subsequently proved the presence of complement-fixing antibodies in 96 of 106 patients with Hashimoto's thyroiditis, 64 of 101 cases of spontaneous myxedema, 37 per cent of patients with thyrotoxicosis, and in a smaller per cent of patients with nontoxic nodular goiter, subacute thyroiditis, and thyroid carcinoma. None of 195 control serums was positive. Anderson and co-authors[39] demonstrated an even higher incidence of complement-fixing antibodies in thyroid diseases, but they had a higher percentage of positive reactions in control serums. The incidence of complement-fixing antibodies in our laboratory is presented in Table II. It is apparent that complement-fixing antibodies occur in multiple types of thyroid disease.

Like the previously described antibodies, complement-fixing antibodies to thyroid cellular substance have been identified as gamma globulins.[11, 39] Roitt and Doniach[11] have shown these antibodies to be organ specific for the thyroid in 87 per cent of positive serums. Belyavin and Trotter[40] have confirmed the organ specificity of the antibody and the antigen. No fixation of complement could be obtained when extracts of liver, spleen, kidney, adrenal, lung, or skin were used as the antigen. The species specificity of the complement-fixing antibody parallels that of the precipitin antibodies, i.e., antibodies to human thyroid extracts react strongly with thyroid extracts of the rhesus monkey and the chimpanzee, but not with those of the rabbit, guinea pig, rat, cow, sheep, pig, or horse.[11]

In the human, the antigen reacting with this complement-fixing antibody is not thyroglobulin, but an intracellular substance found in the microsome fraction of thyroid cells.[11, 39] Belyavin and Trotter[40] extracted $I^{131}$-labeled protein from toxic thyroid glands and determined that the antigen was not an iodinated compound. This antigen also differs from thyroglobulin by being completely precipitated by 36 per cent saturated ammonium sulfate.[41] Thyroglobulin is soluble at this concentration. It is remarkable that the antigen is found in much greater concentra-

tion in glands of patients with thyrotoxicosis and dyshormonogenesis than in the glands of normal individuals or individuals with other types of thyroid disease.[39]

Conflict has arisen over whether the blood group substances present in thyroid extracts give falsely positive complement-fixation reactions. Roitt and Doniach[11] found that a potent anti-A serum gave partial fixation of complement. On this basis they recommended the use of antigen obtained from type O individuals for in vitro testing. Anderson and colleagues,[39] however, were unable to confirm this finding. In our laboratory we have observed instances where serums high in anti-A gave false positive reactions with antigen obtained from individuals of blood group A.[41]

**Complement-fixing antibodies to thyroglobulin.** Two groups of investigators have recently identified a second type of complement-fixing antibody. The antigen that combines with this antibody to fix complement is thyroglobulin. It should be recalled that thyroid cellular substance is the antigen that combines with the previously described complement-fixing antibody. Anderson, Goudie, and Gray[42] found that 9 of 53 patients with Hashimoto's thyroiditis had complement-fixing antibodies to thyroglobulin in their serums. By utilizing immunoelectrophoretic techniques confirmation was obtained that thyroglobulin was the antigen.

Belyavin and Trotter[40] characterized the 2 complement-fixing antibodies. The complement-fixing antibody reacting to thyroglobulin was identifiable in a system using concentrations of weak antigen, but the complement-fixing antibody to thyroid cellular substance was not identified. The latter antibody, however, was identified in a system using weak serum dilutions while the complement-fixing antibody to thyroglobulin was not. This probably reflects the fact that thyroid extracts have high concentrations of thyroglobulin but low concentrations of thyroid cellular substance.

Complement-fixing antibodies to thyroglobulin were looked for in 28 of our serums with a high titer of complement-fixing antibodies to thyrotoxic extracts. In a complement-fixation system, using as the antigen an extract of a nontoxic goiter instead of that from a thyrotoxic gland, all 28 serums gave negative reactions. Sixteen serums were also tested by the complement-fixation technique with the use of purified thyroglobulin as the antigen. No positive reactions were observed indicating that no antibody of this type was present. Further studies are in progress.

**Antibodies demonstrable by the Coons fluorescein technique.** Antibodies have also been identified by application of the Coons technique which permits recognition of antibodies that are fixed to tissue cells. This method uses a conjugate of fluorescein and gamma globulin. White[43] reported staining reactions of human thyroid tissue with fluorescein-tagged gamma globulin taken from the same patient. This patient had Hashimoto's thyroiditis. The staining was primarily of the intrafollicular colloid, i.e., thyroglobulin, although some antigen was present as round globules in the follicular epithelial cells. Beutner and co-authors,[44] by using fluorescein-conjugated goat, anti-rabbit gamma globulin and placing this with rabbit thyroid slices previously incubated with rabbit serum, demonstrated an antigen-antibody complex in the thyroid slices when serums of immunized rabbits were used. Landing,[45] working in conjunction with us, has used this technique extensively. A positive Coons test is obtained in the presence of complement-fixing antibodies or in the presence of agglutinating antibodies. The complement-fixing and agglutinating antibodies cannot be differentiated by this method. In our experience, the staining with both types of antibodies is primarily of the follicle cell cytoplasm.

**Antibodies demonstrated by skin testing.** Ovary and associates[46] described in guinea pigs a passive cutaneous anaphylactoid reaction which demonstrates thyroid specific autoantibodies. These authors have shown that an intradermal injection of serum containing circulating antibodies into guinea pigs reacts locally with homologous thyroid extract injected intravenously. Both organ

and species specificity of the antigen and the antibody were demonstrated since heterologous thyroid extracts and extracts of human kidney and pancreas did not produce a reaction. No reaction occurred when the human serum was incubated with thyroglobulin prior to intradermal injection. This indicates that the antigen is thyroglobulin and not thyroid cellular substance.

Ovary has tested 4 of our serums. Two are strongly positive by both the complement-fixation technique and the agglutinating technique. Two serums are strongly positive only by the complement-fixation technique. The results are given in Table III. They indicate that antibodies measured by the passive cutaneous method are agglutinating and not complement-fixing ones.

Antibodies in humans have also been detected by skin testing. Buchanan and coworkers[47] skin tested 33 patients with thyroid disease and 19 control patients. A sterile thyrotoxic extract containing both thyroglobulin and thyroid cellular substance was injected intradermally as an antigen. Unequivocally positive skin tests were detected in 10 patients. All 10 individuals had positive precipitin tests. However, 5 other patients who did not have skin reactions also had positive precipitin tests. This study did not elucidate whether the reaction was to thyroglobulin or thyroid cellular substance.

In cooperation with Landing, Sutherland, Esselborn, and Pettit, we have used purified thyroglobulin to skin test 2 individuals. These patients had a high titer of complement-fixing antibodies to thyroid cellular substance but no agglutinating antibodies. Intradermal administration of 0.25, 0.50, 1.0, and 2.0 mg. of purified thyroglobulin at weekly intervals failed to give a positive reaction. Serums subsequently obtained at monthly intervals for 9 months were tested by the agglutinating technique. No agglutinating antibodies developed secondary to the injections. This suggests that the individuals studied were not iatrogenically immunized to thyroglobulin. Nevertheless, this technique should be used with caution because of this possibility.

One of these persons was retested with 0.15 ml. of an extract of a thyrotoxic gland. This was sterilized by passing through a Seitz filter. No reaction was observed. This suggests that complement-fixing antibodies are not detected by skin testing, and that Buchanan and co-workers were detecting an antibody against thyroglobulin.

## EFFECT OF TREATMENT

If antibodies are etiologically related to progressive thyroid destruction, the effects of various types of treatment on the appearance, disappearance, and titers of antibodies is of practical concern.

It has been previously reported from this laboratory that agglutinating antibodies spontaneously occur in patients with thyrotoxicosis. These antibodies were found in approximately the same percentage of untreated patients with thyrotoxicosis as those treated medically, surgically, or with radioactive iodine.[28]

The appearance of agglutinating antibodies has been observed during the course of acute thyroiditis.[35] Roitt and Doniach[11] observed the appearance of agglutinating antibodies during the course of subacute thyroiditis in 6 of 12 patients who recovered. In 4 patients the antibodies disappeared with recovery. Unfortunately the type of treatment, if any, was not stated.

These authors[11] and Witebsky and coworkers[36] demonstrated that antibodies may appear following thyroidectomy. Antibody titers decreased, however, in some patients with thyrotoxicosis and Hashimoto's thyroiditis after thyroidectomy.[11]

The administration of 2 grains of desiccated thyroid daily for 2 years did not prevent the development of a positive precipitin reaction in 1 patient with a nontoxic nodular goiter.[11] Similarly, of 14 patients with Hashimoto's thyroiditis who were treated with 2 or more grains of thyroid per day for 1 to 3 years by Doniach and Roitt,[26] half continued to have strong precipitating antibodies in their serum.

It is apparent that the data are conflicting. Better controlled studies are necessary

to determine whether or not therapy can affect the immunization process.

## PATHOLOGY ASSOCIATED WITH AUTOIMMUNIZATION

Witebsky, Rose and co-workers[6, 8] have reported histologic changes in the thyroids of animals immunized with homologous thyroid extracts. Characteristically the acini are small and contain only thin colloid and desquamated cells. The interstitial tissue is densely infiltrated with lymphocytes, plasma cells, and numerous eosinophils. Fibrosis eventually occurs. The pathologic abnormality is roughly proportional to the agglutinating antibody titer.

The similarity of the histologic picture of the thyroid in immunized animals and in patients with Hashimoto's thyroiditis is striking. Thompson[48] studied the pathology of thyroids of patients with nontoxic nodular goiter, toxic goiter, giant cell thyroiditis, and carcinoma. The majority of these individuals had positive precipitin tests. In all instances there was a superimposed histologic picture of Hashimoto's thyroiditis characterized by infiltration with lymphoid tissue and plasma cells and an eosinophilic degeneration of epithelium. Goudie and colleagues[15] also found scattered areas resembling Hashimoto's thyroiditis in the thyroids of supposedly normal individuals who had positive complement-fixing antibodies to thyroid cellular substance. The typical changes are shown in Fig. 2. This picture demonstrates a very localized area of lymphoid infiltration taken from the thyroid of one of our patients who had a nontoxic nodular goiter and circulating antibodies. Occasionally these histologic changes are observed, however, in patients with no detectable circulating antibodies.[48]

## THE CYTOTOXIC FACTOR (ANTIBODY?)

Roitt and Doniach[11] attempted to demonstrate a thyrocytotoxic effect of a serum containing both agglutinating and complement-fixing antibodies. One hundred sixty milliliters of serum were passively transferred into a monkey in divided doses over a period of 7 months. Resection and study of the thyroid gland revealed no histologic abnormality, nor could an antigen-antibody complex be demonstrated by the Coons technique.

Subsequently Pulvertaft and co-authors[30] demonstrated a definite cytotoxic effect of serums from 5 patients with Hashimoto's thyroiditis. Thyroid cells ordinarily grow in tissue culture when normal human serum is used as a substrate. In contrast, serums from these patients with Hashimoto's thyroiditis did not permit growth. Thyroid cells grown normally in tissue culture were destroyed when placed in the serums containing the cytotoxic factor. Pulvertaft has confirmed these findings using serums from 2 of our patients, 1 with Hashimoto's thyroiditis and 1 with spontaneous myxedema.

The absence of agglutinating, precipitating, and complement-fixing antibodies to thyroglobulin in 1 of our serums containing the cytotoxic factor indicates that none of these antibodies is the cytotoxic agent (Table III—C. H.). Both of our patients

**Table III.** Type of antibody

| *Serum of patient* | *Complement-fixing to* | | *Agglutinating (3)* | *Precipitating (4)* | *Passive cutaneous (guinea pig)* (5)* | *Cytotoxic factor (6)* |
|---|---|---|---|---|---|---|
| | *Thyroid cellular substance (1)* | *Thyroglobulin (2)* | | | | |
| M. B. | ++++ | 0 | 1:86,000 | + | + | |
| D. B. | ++++ | 0 | 1: 2,560 | 0 | + | + |
| C. H. | ++++ | 0 | 0 | 0 | 0 | |
| I. H. | ++++ | 0 | 0 | 0 | 0 | + |

*The data in the first 4 columns are correlated with those in column 5 and column 6. Column 5 pertains to the passive cutaneous anaphylactoid reaction in the guinea pig, as measured by Ovary and associates.[46] Column 6 pertains to the thyrocytotoxic factor as measured by Pulvertaft and co-workers.[30] Analysis of these data suggests that (1) the guinea pig skin test measures the same antibody as the agglutinating technique, and (2) the thyrocytotoxic agent is not agglutinating, precipitating, or complement-fixing antibody to thyroglobulin.

with the cytotoxic factor had complement-fixing antibody to thyroid cellular substance, but Pulvertaft and colleagues do not believe this is the destructive agent as heating to 56° C. fails to destroy the antibody but does destroy the cytotoxic effect. Since addition of guinea pig complement does not restore the cytotoxic effect, heating affects the cytotoxic activity in some way other than by destroying complement. In addition, one serum previously studied by Pulvertaft and associates[30] contained the cytotoxic factor but had no complement-fixing antibody. Therefore, cytotoxic factor has been demonstrated in the absence of all currently identified antibodies.

## DISCUSSION

There is little doubt that autoimmunization occurs in thyroid disease. There are, however, many unanswered questions concerning the role autoimmunization plays in the pathologic physiology of thyroiditis, thyrotoxicosis, and other thyroid diseases.

Is autoimmunization an initiating factor in thyroid disease? Experiments in which animals have developed circulating antibodies and/or histologic abnormalities of the thyroid gland following injections of homologous thyroid extracts suggest that autoimmunization could be an initiating factor. It seems unlikely in the human, however, that antigens which are necessary to initiate the immunization process are present outside of the confines of the thyroid, unless (1) there is an antigen elsewhere in the body that is antigenically similar to thyroid extracts, or (2) antigens have been released from their normal confines in the thyroid by an insult to the thyroid such as irradiation, infection, or excessive thyrotropic stimulation. Since there is no evidence at present for cross reactivity between extracts of the thyroid and those of other body organs, one cannot implicate the release of an antigen, with subsequent antibody production, from any source other than the thyroid. The reasonable conclusion is that most instances of thyroid disease autoimmunization, with appearance of currently identifiable circulating antibodies, are the result and not the initiating cause of the disease.

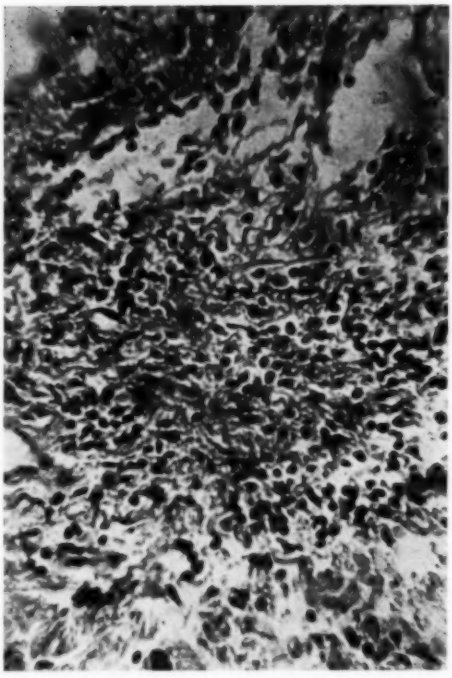


**Fig. 2.** Lymphoid tissue has completely replaced the normal thyroid tissue. This area was one of several very localized areas surrounded by normal thyroid tissue. (This thyroid was removed from a patient with a nontoxic nodular goiter and circulating antibodies.)

One possible exception to this concept must be considered. Placental transfer of thyroid antibodies does occur,[41] and these, if cytotoxic, could induce thyroid disease in the infant in utero. We have observed both complement-fixing antibodies to thyroid cellular substance and agglutinating antibodies to thyroglobulin in several mothers and their respective newborn infants. In 2 instances, antibodies in a given mother and her infant have been associated with cretinism in the baby. In several instances, however, where a mother and her infant had antibodies, the infant had no evidence of disease. This absence of thyroid disease in these latter infants may reflect the present concept that currently identifiable antibodies are not necessarily cytotoxic.

The second question is: What evidence is there that the antibodies which we now identify are cytotoxic? Intimately related to this question is still another: Are these antibodies capable of perpetuating thyroid disease or in altering its characteristics? The answer to the latter question is contingent on the former, and, therefore, these questions may be considered together.

The animal experiments previously discussed in which immunization with thyroid extracts have produced histologic alterations suggest that these antibodies may be cytotoxic. The occurrence of a localized histologic picture of Hashimoto's thyroiditis in the thyroid glands of apparently normal individuals with complement-fixing antibodies also is compatible with the theory that these antibodies are cytotoxic.[18] Possibly, however, the minimal histologic alterations observed in these thyroids may have been present prior to the formation of antibodies. If this is true, the release of antigen is secondary to thyroid disease, and the presence of antibodies is incidental.

Of even greater importance in considering these questions is the high incidence, 35 to 65 per cent, of both complement-fixing and agglutinating antibodies in patients with thyrotoxicosis. Only 10 per cent of patients with thyrotoxicosis, however, eventually develop myxedema.[49] The percentage would be expected to be much higher if these antibodies were cytotoxic to the thyroid. The accumulated evidence suggests that these antibodies are probably not cytotoxic, but merely reflect the presence of thyroid disease.

The question which will undoubtedly receive the most intensive consideration in the near future is: What is the factor that is cytotoxic for thyroid tissue and which has been identified by Pulvertaft and collaborators[30] in the serums of patients with Hashimoto's thyroiditis? This agent which has the ability to destroy thyroid cells may be an antibody, but it probably is not one of those previously identified and discussed in this paper. Evidence for this is that 1 (Table III—C. H.) of the 2 cytotoxic serums, which Pulvertaft tested from this laboratory, had only one antibody, the complement-fixing antibody to thyroid cellular substance. This antibody was not found, however, in 1 cytotoxic serum previously tested.[30]

Conceivably, this cytotoxic agent could be an incomplete or blocking antibody. It has not been possible to demonstrate a blocking antibody to thyroglobulin in the cytotoxic serum of C. H. (Table III). This serum was incubated at a concentration of 1:1 with thyroglobulin coated, tannic acid treated, red blood cells. Serum known to contain agglutinating antibodies was then tested by the agglutinating technique using these red blood cells as the indicator system. The titer of the serum was identical with that observed when normally coated red blood cells were used. In addition, incubation of the serum of C. H. with normal thyroid extract, in a concentration of 3:1, did not decrease the antigenicity of the extract when it was used as the antigen in known positive precipitin reactions. These tests indicate there were no blocking antibodies for thyroglobulin.

Could the cytotoxic agent be a reagin? Pulvertaft and co-authors[30] have suggested this as a possibility. Reagins are heat labile antibodies as is the cytotoxic agent, and reagins are not identifiable by agglutinating, precipitating, or complement-fixing technique. This possibility remains to be verified.

Another question pertains to the detection of the cytotoxic factor: Is there any way to suspect the presence of such an agent other than by in vitro destruction of thyroid cells grown in tissue culture? The presence of antibodies suggests that the cytotoxic factor may be present since the 5 cytotoxic serums reported by Pulvertaft and associates all had either complement-fixing and/or agglutinating antibodies.

Also, the finding of an elevated gamma globulin level, although a relatively nonspecific test, also may suggest the presence of a cytotoxic agent. Of possible significance is the observation that patients with Hashimoto's thyroiditis have previously been noted to have an elevated gamma globulin.[24] The gamma globulin levels in the serums of our

2 patients with cytotoxic factor were 2,200 and 1,700 mg. per cent (normal < 1,500 mg. per cent).

Further investigation is obviously necessary before the interrelation of the autoimmunization and the autodestructive process in thyroid disease can be elucidated. However, the coincident finding of antithyroid antibodies and a cytotoxic factor in some serums of patients with thyroid disease suggests that autoimmunization and autodestruction are intimately related. It is anticipated that information obtained from the studies of thyroid autoimmunization and autodestruction will be productive not only in furthering the understanding of thyroid disease, but also in understanding Addison's disease, idiopathic hypoparathyroidism, orchitis, sympathetic ophthalmoplegia, and other diseases of obscure etiology.

## SUMMARY

The history of autoimmunization and autodestruction in thyroid disease has been reviewed.

Multiple types of antibodies, including agglutinating antibodies, 2 types of complement-fixing antibodies, and precipitating antibodies, can currently be demonstrated in the serums of some patients with various thyroid diseases. These antibodies usually reflect the presence of thyroid disease. It cannot definitely be stated whether these antibodies initiate or perpetuate the disease process.

A cytotoxic factor, other than these antibodies, has been observed in the serums of some patients with Hashimoto's thyroiditis and spontaneous myxedema. The specific identification of this substance and its role in producing or perpetuating thyroid disease remains to be elucidated.

The current concepts relating to the interrelation of autoimmunization and autodestruction in thyroid disease have been discussed.

We would like to express our appreciation to Mr. Joseph O'Neill, Miss Jane Taylor, and Miss Marilyn Kyle for technical assistance, to Dr. Irene Solomon for reviewing the manuscript, and to Miss Mary Carmean for secretarial assistance.

## REFERENCES

1. Kabat, E. A., Wolf, A., and Bezer, A. E.: The Rapid Production of Acute Disseminated Encephalomyelitis in Rhesus Monkeys by Injection of Heterologous and Homologous Brain Tissue With Adjuvants, J. Exper. Med. **85:** 117, 1947.
2. Rivers, T. M., and Schwentker, F. F.: Encephalomyelitis Accompanied by Myelin Destruction Experimentally Produced in Monkeys, J. Exper. Med. **61:** 689, 1935.
3. Morgan, I. M.: Allergic Encephalomyelitis in Monkeys in Response to Injection of Normal Monkey Nervous Tissue, J. Exper. Med. **85:** 131, 1947.
4. Freund, J., Lipton, M. M., and Thompson, G. E.: Aspermatogenesis in Guinea Pig Induced by Testicular Tissue and Adjuvants, J. Exper. Med. **97:** 711, 1953.
5. Freund, J., Lipton, M. M., and Thompson, G. E.: Impairment of Spermatogenesis in the Rat After Cutaneous Injection of Testicular Suspension With Complete Adjuvants, Proc. Soc. Exper. Biol. & Med. **87:** 408, 1954.
6. Rose, N. R., and Witebsky, E.: Studies on Organ Specificity. V. Changes in the Thyroid Glands of Rabbits Following Active Immunization With Rabbit Thyroid Extracts, J. Immunol. **76:** 417, 1956.
7. Witebsky, E., Rose, N. R., Paine, J. R., and Egan, R. W.: Thyroid-specific Autoantibodies, Ann. New York Acad. Sc. **69:** 669, 1957.
8. Witebsky, E., Rose, N. R., Terplan, K., Paine, J. R., and Egan, R. W.: Chronic Thyroiditis and Autoimmunization, J. A. M. A. **164:** 1439, 1957.
9. Anderson, J. R., Goudie, R. B., and Timbury, G. C.: Autoantibodies in Addison's Disease, Lancet **1:** 1123, 1957.
10. Cruikshank, B., and Stuart-Smith, D. A.: Orchitis Associated With Sperm Agglutinating Antibodies, Lancet **1:** 708, 1959.
11. Roitt, I. M., and Doniach, D.: Human Autoimmune Thyroiditis: Serological Studies, Lancet **2:** 1027, 1958.
12. Owen, S. G., and Smart, G. A.: Thyroid Antibodies in Myxoedema, Lancet **2:** 1034, 1958.
13. Doniach, D., Roitt, I. M., and Hudson, R. V.: Auto-antibodies in Thyroid Carcinoma. Letter to the Editor, Lancet **2:** 265, 1958.
14. Stuart, A. E., and Allan, W. S. A.: The Significance of Basement-Membrane Changes in Thyroid Disease, Lancet **2:** 1204, 1958.
15. Goudie, R. B., Anderson, J. R., and Gray, K. S.: Complement-fixing Antithyroid Antibodies in Hospital Patients With Asymptomatic Thyroid Lesions, J. Path. & Bact. **77:** 389, 1959.
16. Papazolu, A.: Contributions à l'étude de la pathologie de la maladie de Basedow, Compt. rend. Soc. biol., Paris **2:** 671, 1911.
17. Hektoen, L., and Schulhof, K.: The Precipitin Reaction of Thyroglobulin, J. A. M. A. **80:** 386, 1923.

18. Hektoen, L., Fox, H., and Schulhof, K.: Specificness in the Precipitin Reaction of Thyroglobulin, J. Infect. Dis. **40:** 641, 1927.
19. Witebsky, E., Rose, N. E., and Shulman, S.: Studies on Organ Specificity. I. The Serological Specificity of Thyroid Extracts, J. Immunol. **75:** 269, 1955.
20. Rose, N. R., and Witebsky, E.: Studies on Organ Specificity. II. Serological Interrelationships Among Thyroid Extracts of Various Species, J. Immunol. **75:** 282, 1955.
21. Shulman, S., Rose, N. R., and Witebsky, E.: Studies on Organ Specificity. III. Ultracentrifugal and Electrophoretic Examinations of Thyroid Extracts, J. Immunol. **75:** 291, 1955.
22. Rose, N. R., and Witebsky, E.: Studies on Organ Specificity. IV. Production of Rabbit Thyroid Antibodies in the Rabbit, J. Immunol. **76:** 408, 1956.
23. Roitt, I. M., Doniach, D., Campbell, P. N., and Hudson, R. V.: Autoantibodies in Hashimoto's Disease, Lancet **2:** 820, 1956.
24. Skillern, P. G., Crile, G. Jr., McCullagh, E. P., Hazard, J. B., Lewis, L. A., and Brown, H.: Struma Lymphomatosa: A Primary Thyroid Failure With Compensatory Thyroid Enlargement, J. Clin. Endocrinol. **16:** 35, 1956.
25. Luxton, R. W., and Cooke, R. T.: Hashimoto's Struma Lymphomatosa. Diagnostic Value of Serum Flocculation Reactions, Lancet **2:** 105, 1956.
26. Doniach, D., and Roitt, I. M.: Auto-immunity in Hashimoto's Disease and Its Implications, J. Clin. Endocrinol. **17:** 1293, 1957.
27. Doniach, D., and Hudson, R. V.: Lymphadenoid Goitre (Hashimoto's Disease). Diagnostic and Biochemical Aspects, Brit. M. J. **1:** 672, 1957.
28. Blizzard, R. M., Hamwi, G. J., Skillman, T. G., and Wheeler, W. E.: Thyroglobulin Antibodies in Multiple Thyroid Diseases, New England J. Med. **260:** 112, 1959.
29. Blizzard, R. M., Hamwi, G., Skillman, T., and Wheeler, W. E.: Circulating Antibodies to Thyroglobulin. A Potential Mechanism in Multiple Thyroid Diseases, Clin. Res. **6:** 404, 1958.
30. Pulvertaft, R. J. V., Doniach, D., Roitt, I. M., and Hudson, R. V.: Cytotoxic effects of Hashimoto Serum on Human Thyroid Cells in Tissue Culture, Lancet **2:** 214, 1959.
31. Goudie, R. B., Anderson, J. R., Gray, K. G., Clark, D. H., Murray, I. P. C., and McNicol, G. P.: Precipitin Tests in Thyroid Disease, Lancet **2:** 976, 1957.
32. Roitt, I. M., Campbell, P. N., and Doniach, D.: The Nature of the Thyroid Autoantibodies Present in Patients With Hashimoto's Thyroiditis (Lymphadenoid Goiter), Biochem. J. **69:** 248, 1958.
33. Korngold, L., VanLeeuwen, G., and Brener, J. L.: The Determination of the Molecular Weight of Different Autoprecipitins in Hashimoto's Disease by a New Immunologic Method, J. Lab. & Clin. Med. **53:** 517, 1959.
34. Rose, N. R.: Thyroid Autoantibodies: Evidence for the Existence of Antibodies of Different Specificities (Abstract), VII. Internat. Congress of Microbiol., 1958.
35. Cline, M. J., Selenkow, H. A., and Brooks, M. S.: Autoimmunity in Thyroid Disease, New England J. Med. **260:** 117, 1959.
36. Witebsky, E., Rose, N. R., and Shulman, S.: The Autoantibody Nature of the Thyroiditis Antibody, and the Role of Thyroglobulin in the Reaction, Lancet **1:** 808, 1958.
37. Rose, N. R., and Witebsky, E.: Studies in Organ Specificity. VI. Cross Reactions of Thyroid Autoantibodies With Thyroids of Other Species, J. Immunol. **83:** 34, 1959.
38. Trotter, W. E., Belyavin, G., and Waddams, A.: Precipitating and Complement Fixing Antibodies in Hashimoto's Disease, Proc. Roy Soc. Med. **50:** 961, 1957.
39. Anderson, J. R., Goudie, R. B., and Gray, K. G.: The "Thyrotoxic" Complement Fixation Reaction, Scottish M. J. **4:** 64, 1959.
40. Belyavin, G., and Trotter, W. R.: Investigations of Thyroid Antigens Reacting With Hashimoto's Sera. Evidence for an Antigen Other Than Thyroglobulin, Lancet **1:** 648, 1959.
41. Chandler, R. W., and Blizzard, R. M.: Unpublished observations.
42. Anderson, J. R., Goudie, R. B., and Gray, K. G.: Complement Fixing Autoantibody to Thyroglobulin in Hashimoto's Disease, Lancet **1:** 644, 1959.
43. White, R. G.: An Immunological Investigation of Hashimoto's Disease, Proc. Roy. Soc. Med. **50:** 953, 1957.
44. Beutner, E. H., Witebsky, E., Rose, N. R., and Gerbasi, J. R.: Localization of Thyroid and Spinal Cord Autoantibodies by Fluorescent Antibody Technic, Proc. Soc. Exper. Biol. & Med. **97:** 712, 1958.
45. Landing, B. H., West, C. D., and Esselborn, V. M.: Detection of Antithyroid Antibodies. Letter to the Editor, J. Clin. Endocrinol. **18:** 792, 1958.
46. Ovary, Z., Randall, H., Witebsky, E., Rose, N. R., Shulman, S., and Metzgar, R.: Thyroid Specific Autoantibodies Studied by Passive Cutaneous Anaphylaxis of Guinea Pig, Proc. Soc. Exper. Biol. & Med. **99:** 397, 1958.
47. Buchanan, W. W., Anderson, J. R., Goudie, R. B., and Gray, K. G.: A Skin Test in Thyroid Disease, Lancet **2:** 928, 1958.
48. Thompson, A. D.: The Pathology of Hashimoto's Disease, Proc. Roy, Soc. Med. **50:** 950, 1957.
49. Eason, J.: The Correlations of Graves' Disease and Thyroiditis, Edinburgh M. J. (Chir.) **35:** 169, 1928.

# *Struma lymphomatosa in children*

## *Report of 12 cases*

**George W. Clayton, M.D.,* and Curtis M. Johnson, M.D.**

HOUSTON, TEXAS

STRUMA lymphomatosa is a condition characterized by a painless goiter in which cellular infiltration and eventual fibrosis are prominent. The etiology is obscure and the pathologic picture varies considerably. It is felt, however, that the variations represent different stages of development of the condition.[1] It occurs most frequently in adults and predominantly in females. There have been sporadic reports of its occurrence in children.[2-14] Although the condition is thought to be rare in childhood, Gribetz, Talbot, and Crawford[15] reported upon 6 children with this condition, stating that these patients made up one-third of all patients with goiter in their clinic. In the past the diagnosis has rarely been made preoperatively for the usual tests of thyroid function such as the determination of protein-bound iodine and of the radioiodine uptake are usually normal. Skillern and colleagues[1] in an exhaustive study of 36 adults with this condition found there was invariably a disparity of greater than 0.5 μg per cent between the protein-bound iodine level and that of butanol-extractable iodine, and there was also a failure of normal response to thyrotropic hormone. Using these tests as criteria for the diagnosis of lymphocytic struma, we have found over the past several years 12 children ranging in age from 6 to 16 years in whom this diagnosis has been made. The following is a report of the clinical, laboratory, and pathologic data on these patients as well as the results of therapy.

*From the Departments of Pediatrics and Physiology Baylor University College of Medicine, and the Junior League Diagnostic Clinic, Texas Children's Hospital, Houston, Texas.*

**Address, Department of Pediatrics, Baylor University College of Medicine, 1200 M. D. Anderson Boulevard, Houston 25, Texas.*

### METHODS AND MATERIALS

Clinical studies were carried out on 12 patients. Six- and 24-hour radioiodine uptakes were measured by counting over the thyroid area with a scintillation counter after oral administration of 5 μc of $I^{131}$ in capsule form. None of the patients had a history of iodine ingestion and none had received desiccated thyroid prior to the studies. Thyrotropic hormone tests were carried out by administering 5 U.S.P. units of thyrotropic hormone and performing 6- and 24-hour $I^{131}$ uptakes the following day. An increase of between 50 and 100 per cent above the control radioiodine uptake was considered to be a normal response and all of the tests were done on consecutive days.

Determination of protein-bound iodine and butanol-extractable iodine was carried out on all patients.*

*Determinations were performed by Bioscience Laboratories.

Successful needle biopsies were carried out in 8 of the patients according to the method of Crile and Vickery.[16] In 1 patient an operative biopsy was performed. In the other 3 patients needle biopsy was either not successful or permission to carry out the procedure was not obtained. These patients were included in the study because of typical clinical and laboratory findings. The sections were stained with hematoxylin and eosin.

## RESULTS

**Clinical manifestations.** A summary of the clinical and laboratory data is presented in Table I. Goiter was the presenting symptom in all of the patients. It produced no symptoms other than a mass or swelling in the neck. In no instance could a history of pain, tenderness, or pressure symptoms be related to the goiter.

In 2 cases there was a family history of goiter. The maternal grandmother and 13-year-old sister of J. A. C. were both reported to have asymptomatic goiters. The mother of G. S. has a goiter proved by biopsy to be struma lymphomatosa.

In all cases the thyroid was at least 5 times normal size and all of them were exceedingly firm with a definite granular feel to palpation. All were diffusely enlarged, although in some instances one lobe was larger than the other. None of the goiters was nodular. A delphian node could be palpated in many instances.

All of the children were clinically euthyroid. There was no significant aberration of growth in any of the children with the exception of J. A. C. This patient, who was 14 years old when studied, manifested what was presumed to be delayed adolescence. This was based on the findings of short stature and infantile genitals. He has subsequently entered puberty and shown a normal growth spurt.

There were 9 females and 3 males. In some instances the goiter was detected as early as 6½ years of age. Six of the patients had a history of onset between 6½ and 7½ years of age.

The known duration of the goiter ranged from 3 months to 3 years. Seven of the children had known goiters for at least 2 years. Presumably, the goiter could have been present in those with a history of short duration much longer than indicated.

**Protein-bound iodine—butanol-extractable iodine.** The protein-bound iodine was determined in all patients and was within the

**Table I.** Summary of patients studied

| Patient | Age at onset (years) | Duration of symptoms | Age at time of diagnosis (years) | Serum iodine studies: Protein-bound iodine (μg %) | Serum iodine studies: Butanol-extractable iodine (μg %) | Serum iodine studies: Disparity between protein-bound iodine and butanol-extractable iodine | Uptake studies: $I^{131}$ uptake prior to thyrotropic hormone | Uptake studies: $I^{131}$ uptake after thyrotropic hormone | Uptake studies: Per cent increase after thyrotropic hormone |
|---|---|---|---|---|---|---|---|---|---|
| 1. J. A. C. | 14 | 3 months | 14 | 8.8 | 5.1 | 3.7 | 27.5 | 36 | 33 |
| 2. C. A. R. | 6.5 | 1 year | 7.5 | 5.7 | 3.3 | 2.4 | 7.6 | 10.9 | 43 |
| 3. L. N. | 9.5 | 1.5 years | 11 | 6.2 | 4.3 | 1.9 | 37 | 42 | 13 |
| 4. N. I. | 7.5 | 2 years | 9.5 | 5.7 | 5.0 | 0.7 | 20 | 33 | 65 |
| 5. D. L. | 6.5 | 6 months | 7 | 7.6 | 6.1 | 1.5 | 47 | 40 | 0 |
| 6. L. R. | 7 | 1 month | 7 | 6.6 | 3.2 | 3.4 | 38 | 43 | 13 |
| 7. D. M. | 8.5 | 2 years | 10.5 | 7.2 | 3.3 | 3.9 | 46 | 49 | 6 |
| 8. D. C. | 7 | 2 years | 9 | 8.5 | 3.2 | 5.3 | 24 | 17 | 0 |
| 9. F. T. | 12 | 2 years | 14 | 5.3 | 4.8 | 0.5 | 39 | 43 | 10 |
| 10. G. S. | 7.5 | 2 years | 9.5 | 4.7 | 2.9 | 1.8 | 21.4 | 39.6 | 85 |
| 11. D. M. | 13 | 3 years | 16 | 6.8 | 4.9 | 1.9 | 34 | 32 | 0 |
| 12. E. S. | 11 | 2 years | 13 | 5.2 | 3.5 | 1.7 | 29 | 28 | 0 |

range of normal values (4 to 8 μg per cent) in all but 2 instances. These, J. A. C. and D. C., were slightly elevated. The average protein-bound iodine was 6.5 μg per cent with a range of 4.7 to 8.8 μg per cent (Fig. 1).

The butanol-extractable iodine ranged from 2.9 to 6.1 μg per cent with an average of 4.13 μg per cent. Five of the patients had values below the accepted minimal level (normal range 3.5 to 7.5 μg per cent). Of these, only one had a value below 3 μg per cent. None of the patients had elevated values.

There was a greater than normal difference between the protein-bound iodine and butanol-extractable iodine in all but 1 patient. The average disparity was 2.39 μg per cent with a range of 0.5 to 5.3 μg per cent.

**$I^{131}$ uptake and thyrotropic hormone studies.** Studies of the uptake of $I^{131}$ by the thyroid were carried out on all patients. All but 1 patient had normal uptake values (Table I). This patient also had a low uptake following thyrotropic hormone stimulation. The uptakes ranged from 7.6 to 47 per cent with an average value of 30.8 per cent (Fig. 2).

After administration of thyrotropic hormone, $I^{131}$ uptakes ranged from 10.9 to 49 per cent with an average of 34.4 per cent. Only 2 patients showed greater than a 50 per cent increase in $I^{131}$ uptake above control values, and none showed a rise as great as 100 per cent. Four patients failed to show stimulation of the thyroid $I^{131}$ uptake after administration of thyrotropic hormone.

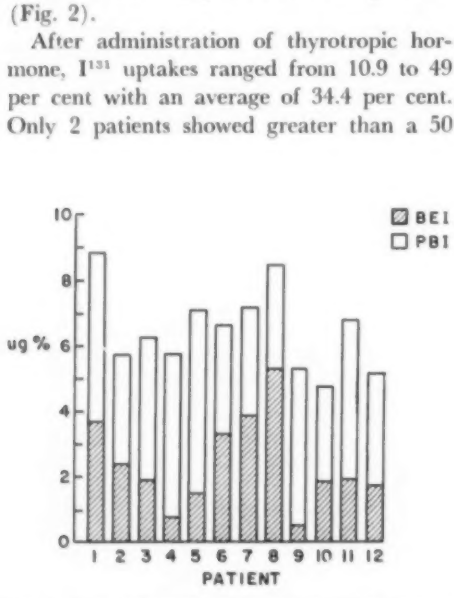



**Fig. 1.** Protein-bound iodine and butanol-extractable iodine determinations.

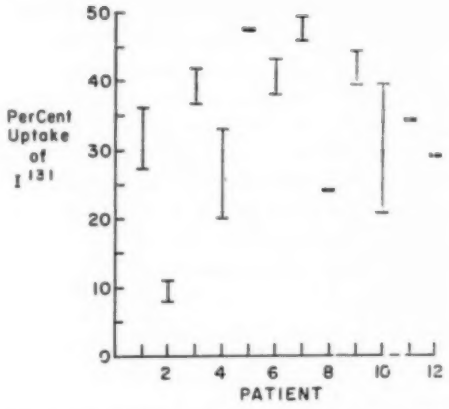



**Fig. 2.** $I^{131}$ uptake before and after administration of thyrotropic hormone.

## PATHOLOGY

The thyroid biopsies uniformly showed extensive infiltration of lymphocytes between the follicles (Fig. 3). The thyroid cells were hyperplastic and the follicles varied in size, some being quite small. Colloid was frequently dense and sometimes scant, and fibrosis, if present, was usually mild. This represented the hyperplastic type as none of the biopsies showed the oxyphilia characteristic of the Hashimoto type.[1] The lack of oxyphilia suggests an earlier form of the disease and is characteristically seen in younger patients.

## TREATMENT

Treatment of all patients consisted of U.S.P. desiccated thyroid in doses of 2 or 3 grains daily. We gave doses of 2 grains to those patients who were prepuberal and 3 grains to those who were puberal. A prompt decrease in the size of the thyroid occurred with therapy; in 7 of 8 patients the thyroid was no longer palpable 6 months after institution of therapy. The patients with a long history of goiter required a longer period for

its disappearance. In addition, if the gland was very firm, response to thyroid therapy seemed slower than in some of the patients with smaller and less firm goiters. In one patient, L. R., with a history of goiter of only a few weeks, treatment with thyroid resulted in its prompt disappearance. However, this patient neglected to take her medication for several weeks and the goiter promptly returned. Upon resumption of treatment the goiter again disappeared. In none of the other patients, as far as is known, has therapy been discontinued.

## OTHER STUDIES

Determinations of cholesterol were found to be within the range of normal in all patients. In a few cases in which plasma protein patterns were determined there was an elevation of the beta globulin.

Thiocyanate tests were made in 2 cases and revealed no discharge of radioiodine following administration of thiocyanate.

## DISCUSSION

It is felt that the studies performed on these patients represent an adequate and practical means of diagnosis of struma lymphomatosa in children.

Although other conditions occur in which there is painless enlargement of the thyroid gland, there are few which have the clinical and laboratory findings observed in the patients described above. Patients with subacute and acute thyroiditis usually fail to respond to thyrotropic hormone,[17, 18] but characteristically these 2 conditions, which are rare in children, have a low uptake of radioiodine and the goiter is painful. Mild enzymatic defects in the synthesis of thyroxine may occur, resulting in compensatory enlargement of the thyroid, but without or with only mild hypothyroidism.[19] These goiters may be firm but almost invariably show a marked increase in trapping of radioiodine.

Carcinoma of the thyroid in childhood usually is characterized by a firm nodule or nodularity in the region of the thyroid. In this series of patients with struma lymphomatosa no nodules were felt. In carcinoma

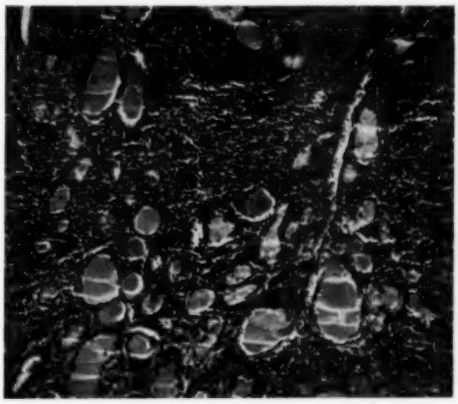


**Fig. 3.** Section of thyroid from patient (L. N.). (Hematoxylin and eosin. × 95; reduced ⅓.)

the unaffected thyroid tissue responds normally to thyrotropic hormone stimulation.

"Iodine" goiters are occasionally seen. These patients usually give a positive history of ingestion of iodine, however, and the uptake of radioiodine is low. Natural goitrogens are rare and the history usually fails to reveal their ingestion.

"Adolescent" goiter is thought to occur as a result of increased need for thyroid hormone during puberty and is said to be rare in areas where there is no iodine deficiency.[14] In addition, tests of thyroid functions are normal.

Although the cause and precipitating factors of struma lymphomatosa are unknown there is increasing evidence that it is probably a primary failure of the thyroid with compensatory hyperplasia due to thyrotropin stimulation.[1] This, it is felt, accounts for the failure of a normal response to stimulation by administered thyrotropic hormone. In addition, the consistent finding of an abnormal disparity of protein-bound iodine and butanol-extractable iodine may well indicate the presence of abnormal iodinated compounds in the circulation as a result of destruction or dysfunction of the thyroid. Owen and McConahey[20] have demonstrated an iodinated protein in the serum of patients with struma lymphomatosa which may be thyroglobulin. The observations of Witebsky

and associates[21] and of Doniach and Roitt[22] suggested that in struma lymphomatosa thyroglobulin was released into the circulation and serves as a foreign protein leading to the production of antibodies. In many patients with struma lymphomatosa there is a high titer of antibodies. However, recent studies[23, 24] have indicated that these antibodies are found in a variety of thyroid diseases. It is true that the method for determining the butanol-extractable iodine would exclude thyroglobulin, but would also exclude such compounds as the tyrosines.

The differences in the pathologic findings in the children of this series in contrast to those in adults is of considerable interest. Whereas only a few adults with this condition are found to have the "hyperplastic type" of struma, all of the children in this group who were subjected to biopsy showed this type. It is the opinion of some observers[1] that these differences are only of degree and that without therapy the thyroid eventually may become decompensated and the patient becomes hypothyroid. As the process apparently is of long duration it is doubtful that children with this condition will be found who are hypothyroid.

It is the authors' opinion that an operation is not necessary in these cases and that the condition may usually be diagnosed without such a procedure. A prompt decrease in the size of the gland has been observed with therapy with desiccated thyroid and, in most instances where long-term follow-up has been possible, the thyroid is no longer palpable.

## SUMMARY

The clinical, laboratory, and pathologic findings in 12 children with struma lymphomatosa have been presented. Laboratory studies showed an abnormally low response to thyrotropic hormone. The difference between the levels of protein-bound iodine and of butanol-extractable iodine was greater than normal in all patients but one. The pathologic studies obtained by needle biopsy and in one instance by an operative technique were described. The biopsies revealed the "hyperplastic type" of struma lymphomatosa in all instances. Possible explanations of the laboratory and pathologic findings were discussed, and the therapeutic results of treatment with desiccated thyroid described.

The authors wish to thank Dr. Alfred Leiser for his help in carrying out these studies.

## REFERENCES

1. Skillern, Penn G., Crile, G., Jr., McCullagh, E. P., Hazard, J. B., Lewis, L. A., and Brown, H.: Struma Lymphomatosa: Primary Thyroid Failure With Compensatory Thyroid Enlargement, J. Clin. Endocrinol. **16:** 35, 1956.
2. McGuillan, A. S.: Thyroiditis, in Transactions of the Third International Goiter Conference and the American Association for the Study of Goiter, Washington, D. C., Sept. 12 to 14, 1938, Portland, Oregon.
3. Bogart, L. M.: Discussion of Lerman, J. A.: Hashimoto's Struma, in Transactions of the Third International Goiter Conference and the American Association for the Study of Goiter, Washington, D. C., September 12 to 14, 1938, Portland, Oregon.
4. Hellwig, C. A.: Lymphadenoid Goiter, Arch. Path. **25:** 838, 1938.
5. Joll, C. A.: Pathology, Diagnosis and Treatment of Hashimoto's Disease (Struma Lymphomatosa, Brit. J. Surg. **27:** 351, 1939.
6. Schilling, J. A.: Struma Lymphomatosa, Struma Fibrosa and Thyroiditis, Surg. Gynec. & Obst. **81:** 533, 1945.
7. Parmley, C. C., and Hellwig, C. A.: Lymphadenoid Goiter: Its Differentiation From Chronic Thyroiditis, Arch. Surg. **53:** 190, 1946.
8. Lasser, R. P., and Grayzel, D. M.: Subacute Thyroiditis, Struma Fibrosa, Struma Lymphomatosa: Clinical-Pathological Study, Am. J. M. Sc. **217:** 518, 1949.
9. Davison, T. C., and Letton, A. H.: Hashimoto's Disease, J. Clin. Endocrinol. **9:** 980, 1949.
10. Lindsay, S., Dailey, M. E., Friedlander, J., Yee, G., and Soley, M. H.: Chronic Thyroiditis: Clinical and Pathological Study of 354 Patients, J. Clin. Endocrinol. **12:** 1578, 1952.
11. Davis, H. C., and Hanske, E. A.: Hashimoto's Disease in Childhood, A. M. A. Am. J. Dis. Child. **90:** 173, 1955.
12. Lyday, R. O.: Struma Lymphomatosa (Hashimoto's Disease) in Children, Am. J. Surg. **94:** 417, 1957.
13. Farber, S., and Craig, J. M.: Clinical Pathological Conference: The Children's Medical Center, Boston, Massachusetts, J. Pediat. **53:** 494, 1958.
14. Wilkins, Lawson: The Diagnosis and Treat-

ment of Endocrine Disorders in Childhood and Adolescence, ed. 2, Springfield, Ill., 1957, Charles C Thomas, Publisher.
15. Gribetz, Donald, Talbot, Nathan B., and Crawford, John D.: Goiter Due to Lymphocytic Thyroiditis (Hashimoto's Struma), New England J. Med. **250:** 555, 1954.
16. Crile, G., Jr., and Vickery, A. L.: Special Uses of Silverman Biopsy Needle in Office Practice and at Operation, Am. J. Surg. **83:** 83, 1952.
17. Robbins, Jacob, Roll, J. E., Trunnell, J. B., and Rawson, R. W.: The Effect of Thyroid Stimulating Hormone in Acute Thyroiditis, J. Clin. Endocrinol. **11:** 1106, 1951.
18. McConahey, W. M., and Keating, F. R., Jr.: Radio-iodine Studies in Thyroiditis, J. Clin. Endocrinol. **11:** 1116, 1951.
19. Clayton, George W., Smith, J. Darrel, and Leiser, Alfred: Familial Goiter With Defect in Intrinsic Metabolism of Thyroxine Without Hypothyroidism, J. PEDIAT. **52:** 129, 1958.
20. Owen, C. A., and McConahey, W. M.: An Unusual Iodinated Protein of the Serum in Hashimoto's Thyroiditis, J. Clin. Endocrinol. **16:** 1570, 1956.
21. Witebsky, E., Rose, N. R., Terplan, K., Paine, J. R., and Egan, R. W.: Chronic Thyroiditis and Auto-immunization, J. A. M. A. **164:** 1439, 1957.
22. Doniach, D., and Roitt, I. M.: Auto-immunity in Hashimoto's Disease and Its Implications, J. Clin. Endocrinol. **17:** 1293, 1957.
23. Blizzard, R. M., Hamwi, G. J., Skillman, T. G., and Wheeler, W. E.: Thyroglobulin Antibodies in Multiple Thyroid Diseases, New England J. Med. **260:** 112, 1959.
24. Cline, M. J., Selenkow, H. A., and Brooke, M. S.: Auto-immunity in Thyroid Disease, New England J. Med. **260:** 117, 1959.

# *Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: an example of hormonal overlap in pituitary feedback*

**Judson J. Van Wyk, M.D.,* and Melvin M. Grumbach, M.D.**

CHAPEL HILL, N. C.

A CARDINAL manifestation of untreated juvenile hypothyroidism is failure to grow and mature at a normal rate. Facial bones retain their infantile features, the dentition is retarded, and the lag in skeletal maturation is usually as great or greater than that in linear growth. As in other endocrine disturbances, the level of sexual development of children with juvenile hypothyroidism usually corresponds approximately with the bone age. The present paper defines a syndrome associated with juvenile hypothyroidism in which certain aspects of sexual development are advanced beyond other parameters of maturation.

*From the Departments of Pediatrics, University of North Carolina School of Medicine, Chapel Hill, North Carolina, and the College of Physicians and Surgeons, Columbia University, and the Babies Hospital, New York, New York.*

*Research on endocrine function in children is supported in each institution by grants from the National Institutes of Health, United States Public Health Service.*

**John and Mary R. Markle Scholar in Medical Science. Address, Department of Pediatrics, The School of Medicine, The University of North Carolina, Chapel Hill, N. C.*

Three girls with untreated primary myxedema presented with precocious menstruation, development of the breasts, and galactorrhea, although pubic hair was absent and other indices of maturation were retarded. One patient was abnormally pigmented. These abnormalities rapidly disappeared when thyroid was administered. In the 2 older girls, normal menarche occurred subsequently when the appropriate maturational level had been reached. In all 3 cases there was enlargement of the sella turcica and evidence that the symptoms were due to increased secretion of pituitary hormones. Enlargement of the pituitary above the sella was demonstrated in 1 patient. Another patient developed transient diabetes insipidus. In all 3 patients the sella turcica became demineralized and enlarged further after treatment with thyroid hormone was instituted. After a more prolonged period of euthyroidism, the sella remineralized and in 2 patients a striking decrease in the size of the pituitary fossa was observed. The pituitary fossa in the third patient underwent a relative decrease in size over a 6-year period

when compared with standards of normality for age and stature.

The apparently inappropriate secretion of multiple heterologous pituitary hormones in response to a single target gland deficiency, and in turn their inhibition by the administration of a single target gland hormone, so clearly demonstrated in these prepubescent children, suggests that a similar mechanism may account for a variety of phenomena commonly observed in other clinical conditions. This feature of the pituitary feedback mechanism has received scant attention in the clinical literature.

The original patient was studied jointly by the authors while serving as fellows under Dr. Lawson Wilkins in the Harriet Lane Home Endocrine Clinic. Subsequently, each of the authors has studied a similar case. It is therefore appropriate to bring these observations together in this publication honoring Dr. Wilkins.

## CASE REPORTS

**Case 1** (C. B., H.L.H. B06997). This $8\frac{8}{12}$-year-old white girl was referred to the Harriet Lane Home Endocrine Clinic in 1953 because of precocious menstruation associated with menorrhagia. The mother had a goiter and the oldest sister had taken thyroid and iodine at some time in the past. Five other older siblings were well and there was no family history of precocious puberty or other endocrine disturbance.

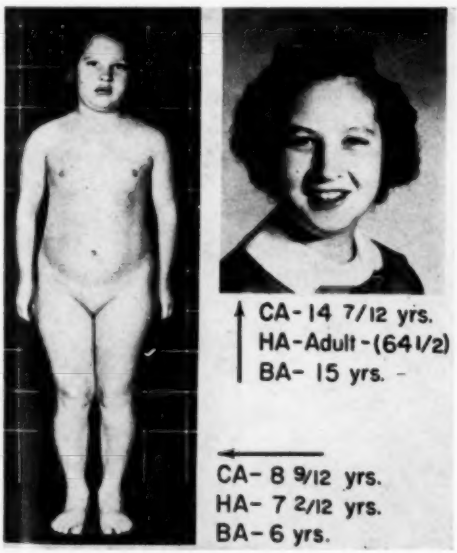



Fig. 1. Photographs (Case 1) taken prior to institution of treatment and about 6 years later. CA = chronologic age; HA = height age; BA = bone age.

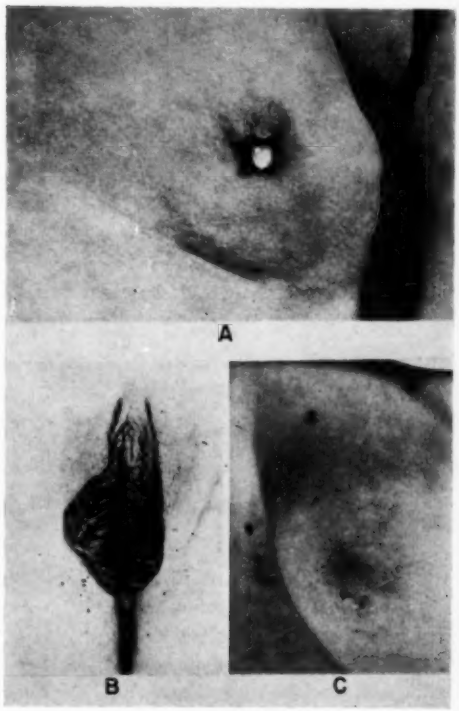



Fig. 2, *A*. Breast showing secretion of colostrum (Case 1). *B*, External genitals (Case 1) demonstrating marked hyperplasia of labia minora. *C*, Left breast (Case 2) showing secretion of colostrum. Several hyperpigmented nevi are also visible.

The patient was a bright child whose developmental milestones had been normal and medical history uneventful. Six months before admission she began to develop puffiness in the face and to gain weight rapidly, although her appetite had diminished. She continued to do well in school. Two months before admission a white mucoid vaginal discharge was noted and 1 month before admission she developed vaginal bleeding which lasted 9 days. Thirteen days before admission

**Table I**

| *Date* | *Area of sella* (sq. mm.)* | *Range of normal (5 to 95%)* | |
|---|---|---|---|
| | | *Stature (sq. mm.)* | *Chronological age (sq. mm.)* |
| Nov. 30, 1953 | 93.8 | 34 to 76 | 42 to 84 |
| Feb. 27, 1954 | 96 | 36 to 76 | 42 to 84 |
| Feb. 22, 1955 | 99 | 42 to 78 | 42 to 84 |
| Oct. 2, 1959 | 100+ | 52 to 98 | 58 to 100 |

*We are indebted to Dr. Ernest Wood, Professor of Radiology, University of North Carolina School of Medicine, for recalculating the sagittal areas of the pituitary fossae by the method of Silverman[1] to permit comparison with appropriate standards of normality. These areas are smaller than those given in the case reports which were calculated by the method of Hare.[2]

she had another episode of profuse vaginal bleeding which lasted 5 days. She had had no headaches, visual disturbances, polyuria, polydipsia, or other symptoms which could be related to an endocrine disturbance.

On physical examination the pulse was 92 per minute and the blood pressure 94/70. The child was small and obese with marked pallor and obvious myxedema (Fig. 1). The height was 47.5 inches (height for 7¼ years), and the weight was 73.5 pounds (average for height, 48.3 pounds). The skin was mottled and dry but not excessively cold. The facial features were immature, giving the impression of a child younger than the stated age. The dental age was 7 years. The thyroid was not enlarged. The glandular tissue which was palpable in each breast was poorly delineated, although the areolae and nipples showed a pronounced estrogenic effect. A copious secretion of opalescent colostrum was easily expressed from each breast (Fig. 2, *A*). There were a few dark pubic hairs, about 6 in number and none longer than 1 inch. There was marked hypertrophy of the labia minora which were about 5 cm. long (Fig. 2, *B*). The vulvar mucous membranes were thickened, pale, and corrugated. The clitoris was not enlarged. The labia majora were slightly pigmented. Rectal examination under anesthesia revealed a uterus of adult size and a cystic mass about 6 cm. in diameter in the right adnexa. The neurological examination was within normal limits. Both optic discs were somewhat blurred, but there was no evidence of papilledema. The visual fields were full by careful confrontation.

*Laboratory examination.* The hemoglobin was 7.4 Gm. per 100 ml. The urine had a specific gravity of 1.020 and was otherwise negative. The bone age was 6 years with no evidence of epiphyseal dysgenesis. An x-ray film of the skull (Fig. 3 and Table I) revealed that the sagittal area of the sella as determined by the method of Hare[2] was between 100 and 150 sq. mm. There was no evidence of destruction of the floor, although the posterior portion was poorly mineralized. X-rays of the chest were normal.

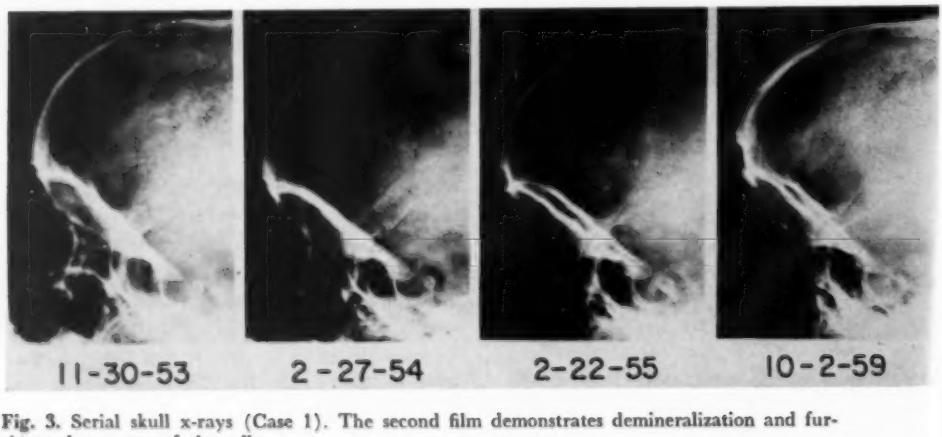



**Fig. 3.** Serial skull x-rays (Case 1). The second film demonstrates demineralization and further enlargement of the sella.

The serum cholesterol was 820 mg. per cent and the protein-bound iodine was 0.8 μg per 100 ml. Twenty-four hours following the intravenous administration of 32 μc of $I^{131}$, only 1.6 counts per second were recorded over the thyroid gland by a Geiger-Müller tube. The test was repeated with an identical amount of radioactive iodine after the patient had received 12.5 mg. of Armour thyrotropin intermuscularly every 12 hours for 6 doses. The 24-hour uptake at this time was 12.9 counts per second. Although these measurements were not expressed then as percentage uptake, it is apparent that there was only a minute accumulation of $I^{131}$ by the thyroid gland and only a slight increase after stimulation with thyrotropic hormone.

Urinary 17-ketosteroids were 2.1 mg. per 24 hours, and urinary estrogens determined fluorometrically were 10 μg per 24 hours. A frog test for chorionic gonadotropin was negative. The follicle-stimulating hormone assay was negative at a level of 6.6 M.U. per 24 hours. The vaginal smear revealed full estrogen effect.

*Course.* Because of the presence of the adnexal mass, abdominal laparotomy was performed and both ovaries were found to contain multiple cysts, some of which were quite large and multiloculated. Each ovary measured 7.5 by 5 cm. These cysts were resected (Fig. 4). Dr. Howard W. Jones interpreted the microscopic findings in the resected cysts as follows: "There are a very few primordial follicles and one must look very hard to find any at all. There is no thickening of the capsule nor is there sub-capsular fibrosis. The linings of the cysts are composed mostly of single layers of cells, but in some instances two or three layers are piled up. These cells do not appear to be actively secretory. The stroma is rather sparse and there may be some suggestion of formation of lutein cells, but this effort is not tremendously impressive. Several of the cysts seem to be regressing and one could probably speak of them as being atretic." Biopsy of the endometrium showed interval nonsecretory epithelium.

The patient was placed on thyroid therapy beginning with ½ grain daily and gradually increasing the dose to 2½ grains a day. She had another menstrual period a few days after the operation, but no further recurrence until 3½ years later when she was 12 years old. The patient did not return for follow-up and was not seen again until recently when, at the age of 14 7/12, she was re-examined by one of the authors in her home town. In the meantime, she had continued to take 2½ grains of desiccated thyroid daily and had been carefully observed by her family doctor.* The galactorrhea had ceased and the breasts had become smaller shortly after she had become euthyroid. When she was 12 years old she developed regular menses at 28-day intervals and in recent months these have been accompanied by cramps. Axillary hair, pubic hair, and normal development of the breasts had appeared almost simultaneously with the true menarche. There had been occa-

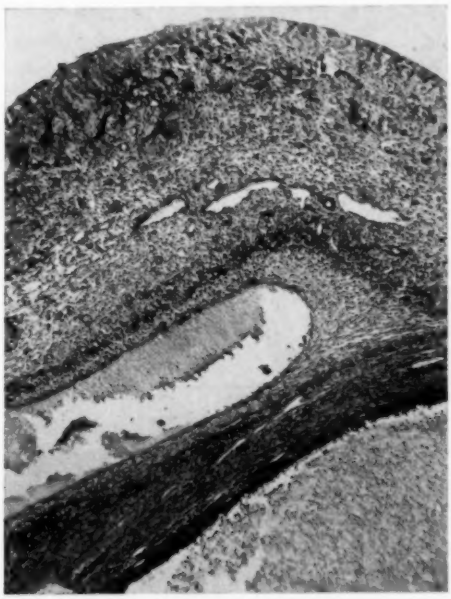


**Fig. 4.** Photomicrograph of the resected portion of ovary (Case 1). Note the paucity of primordial follicles. ×75; reduced approximately ¼.

*We are indebted to Dr. R. C. Hughes of Rocky Mount, Virginia, for his cooperation in arranging this examination and making available to us his office records.

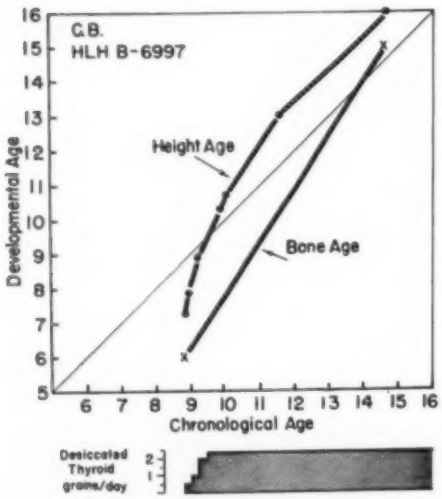



Fig. 5. Growth chart (Case 1).

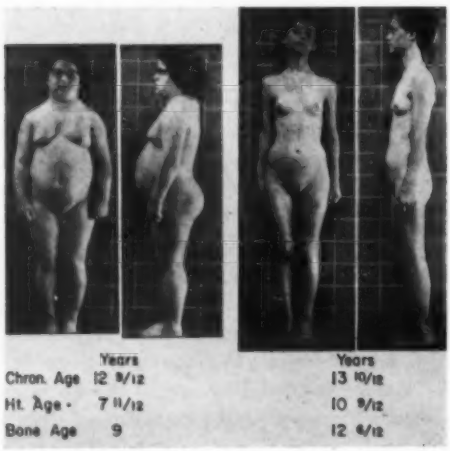



Fig. 6. Photographs (Case 2) taken prior to treatment and 13 months later.

sional mild headaches, but no signs or symptoms suggestive of a pituitary tumor.

At the time of re-examination the patient was euthyroid and had developed into an attractive, somewhat obese teen-ager. Her height was 5 feet 4 inches and her weight 150 pounds (Fig. 5). The obesity was generalized. No secretion could be expressed from the breasts. Fundi and visual fields by confrontation were normal. Repeat skull x-rays revealed that the sella had become better mineralized although by the calculations of Silverman[1] it was slightly larger than it had been 6 years previously (Fig. 3). When compared with standards for stature and age the sella now fell within the upper range of normal, whereas previously it had been greatly enlarged by whatever criterion was used. The bone age was now 15 years.

**Case 2.** (M. W., N.C.M.H. 4-28-63). This $12\frac{8}{12}$-year-old white girl was admitted to the North Carolina Memorial Hospital in April, 1956, because of excess weight gain, failure to grow, and profuse vaginal bleeding. There was no family history of sexual precocity or endocrine disturbance except for the presence of a goiter in an aunt. At the age of 4 years the patient developed a "marble sized" goiter in her neck. She experienced normal growth and development and was considered a bright child until the age of 8 when her linear growth nearly ceased and she rapidly gained weight in spite of a diminished food intake. Progressively she developed puffiness of the features, constipation, pallor, and intolerance to cold. Review of the systems revealed that the child had had no headaches, but for the past 2 years she had had difficulty in seeing the blackboard and this had improved after glasses were prescribed. There had been no polyuria nor polydipsia. She continued to get excellent grades in her schoolwork, although there was a marked change in her habits from those of an active child to those of a sedentary one.

At the age of $11\frac{10}{12}$ years she had her first menstrual period which lasted 5 days and was accompanied by profuse bleeding. At this time her breasts had enlarged, although no pubic hair had appeared. In the subsequent 10 months she had 3 additional periods at irregular intervals; the last one had occurred 5 weeks prior to admission and had lasted 14 days. For the preceding year pigmentation had been noted in the creases of the skin and her nevi had darkened noticeably. For several months she had been embarrassed by mammary secretions which stained her dresses at school.

On physical examination the pulse was

76, and the blood pressure was 115/90. The height was 48.7 inches (height for 8 years), and the weight was 83 pounds (average for height, 51 pounds).

The child was both obese and myxedematous with puffy rolls around the eyes, neck, and abdomen, and a "buffalo hump" which was reminiscent of that seen in Cushing's syndrome (Fig. 6). The extremities were cold, and the pallid skin was dry and scaly. There was deep brownish pigmentation in the axillae and over the elbows, knees, and back of the neck. There was no pigmentation on the buccal membranes, but brown pigmentation was present in the vulvar mucosa. Numerous black, darkly pigmented nevi were present on the trunk. The thyroid isthmus was enlarged (3 by 1.5 cm.) but smooth and very firm in consistency. The dental age was approximately 8 years with extensive caries. Examination of the eyes revealed indistinct disc margins due to a high degree of myopia, but there was no papilledema and the visual fields were full by both confrontation and perimetry.

The sexual development was striking.

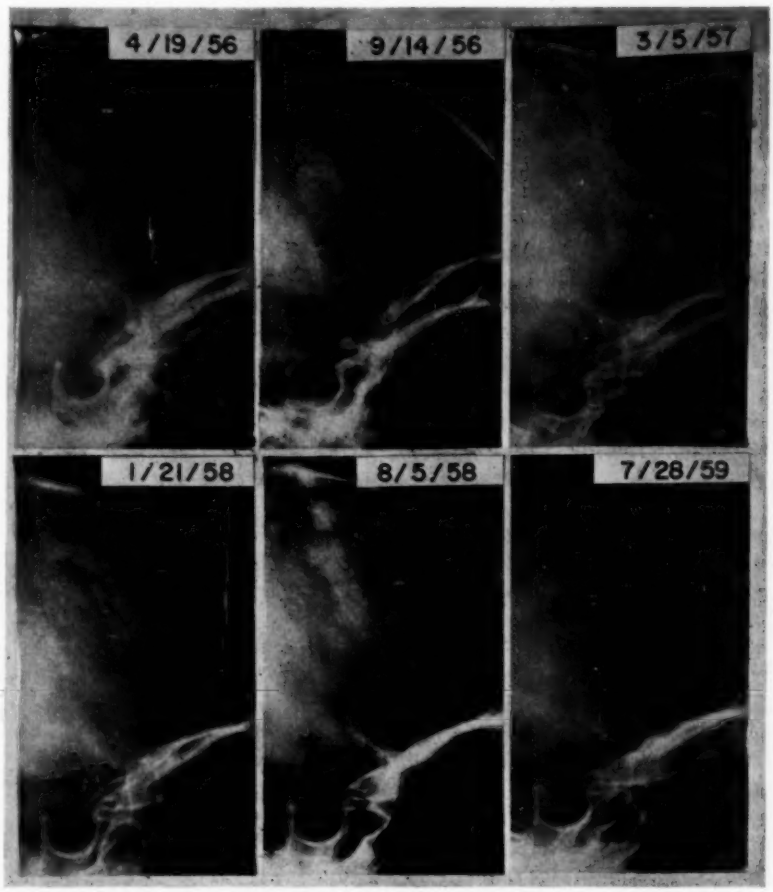



**Fig. 7.** Serial skull x-rays (Case 2) on April 19, 1956, prior to treatment; Sept. 14, 1956, period of increased intracranial pressure, separation of the coronal sutures, and demineralization of the sella and calvarium; March 5, 1957, period of diabetes insipidus. It will be noted that the area of the sella is decreased in subsequent roentgenograms.

**Table II**

| Date | Area of sella* (sq. mm.) | Range of normal (5 to 95%) | |
|---|---|---|---|
| | | Stature (sq. mm.) | Chronological age (sq. mm.) |
| April 19, 1956 | 88.4 | 34 to 76 | 52 to 98 |
| Sept. 14, 1956 | 93.8 | 36 to 78 | 52 to 98 |
| March 5, 1957 | 93.8 | 42 to 78 | 56 to 100 |
| Jan. 21, 1958 | 85.4 | 42 to 82 | 58 to 100 |
| Aug. 5, 1958 | 81.4 | 44 to 86 | 58 to 100 |
| July 28, 1959 | 81.4 | 46 to 88 | 60 to 104 |
| Dec. 21, 1959 | 81.4 | 46 to 92 | 64 to 106 |

*We are indebted to Dr. Ernest Wood, Professor of Radiology, University of North Carolina School of Medicine, for recalculating the sagittal areas of the pituitary fossae by the method of Silverman[1] to permit comparison with appropriate standards of normality. These areas are smaller than those given in the case reports which were calculated by the method of Hare.[2]

There was no pubic or axillary hair. The breasts were conical and embedded in fat, but a large amount of duct tissue was palpable bilaterally. The areolae and Montgomery's follicles were well estrinized. On gentle pressure there was a copious secretion of yellow, clear fluid in which was suspended some white, stringy material (Fig. 2, *C*). Despite the absence of pubic hair, the labia minora were very well developed and thickened. The vulvar mucosa was pale and an abundant mucoid secretion was present. On rectal examination the uterus was enlarged to at least adult size, but no adnexal masses could be palpated. A detailed neurological examination revealed no abnormalities except for a delayed relaxation phase of the deep tendon reflexes.

*Laboratory.* An x-ray film of the skull revealed a sella which was slightly enlarged in its sagittal area and more globular than average in a child of this age, although no bony erosion could be discerned (Fig. 7 and Table II). The bone age was 8 years with epiphyseal dysgenesis noted in the patella and greater trochanters. The hemoglobin was 9.5 Gm. per 100 ml. and the white blood count 7,600 per cubic mm. with a normal differential count. Urinalysis revealed a specific gravity of 1.013, with no significant abnormalities. Serum electrolytes, calcium, phosphorus, total protein, and albumin were normal. The alkaline phosphatase was 2.6 Bodansky units, and total cholesterol content 434 mg. per cent. The protein bound iodine was 1.8 μg per 100 ml. The 24-hour excretion of 17-ketosteroids was 1.7 mg. and of 17-ketogenic steroids 2.3 mg. Three days later 25 units of ACTH was infused over a 6-hour period and the 17-ketosteroids rose to 7.3 mg. and the 17-ketogenic steroids to 17.1 mg. Assay of a 24-hour urine for gonadotropic hormone content revealed less than 6 M.U. The thyroidal uptake of radioactive iodine was less than 6 per cent and it did not rise following the administration of 12.5 units of TSH every 12 hours for a total of 4 doses.

A vaginal smear revealed greater than 60 per cent cornification and an endometrial biopsy revealed a predominantly secretory pattern. A biopsy of the thyroid revealed the characteristic picture of Hashimoto's struma (Fig. 8). A smear of the breast secretion revealed small numbers of neutrophils, lymphocytes, and finely vacuolated macrophages consistent with cystic hyperplasia.

*Course.* The child was discharged from the hospital on 12.5 μg triiodothyronine daily and followed regularly in the clinic where the triiodothyronine was quickly increased to 75 μg daily. She rapidly regained the euthyroid status. There were no further menstrual periods and the galactorrhea disappeared within a few weeks.

Four and one-half months after the institution of treatment she had grown 2 inches and had lost 5 pounds. She had had no headaches, no nausea, and no vomiting. There had been a striking increase in her activity and change in her appearance. The breasts were smaller and the pigmentation had diminished markedly. The thyroid isthmus was no longer enlarged. The blood pressure was 110/70, and the pulse rate was 94. At this time, in the course of a routine outpatient examination, she was found to have papilledema of 3 diopters and was readmitted to the hospital for reinvestigation.

During the next 24 hours hemorrhages appeared in the optic fundi. There was a

cracked-pot sound on percussion of the skull, although the patient remained alert and free of symptoms which could be related to these findings. Careful re-examination of the visual fields at this time revealed no constriction of the field perimeters, but there was a decrease in visual acuity and a significant enlargement of the blind spot to 22 mm. in diameter. Radiologic examination of the skull revealed spreading of the sutures and diffuse demineralization of the calvaria and the base of the skull including the floor of the sella. There had been some further enlargement of the pituitary fossa although there was no definite break in the sellar outline (Fig. 7).

Further laboratory studies at this time revealed a hemoglobin of 13 Gm. per 100 ml., white blood count of 7,000, serum calcium of 10.7 mg. per cent, phosphorus of 4.1 mg. per cent, blood urea nitrogen of 18 mg. per cent, and an $I^{131}$ uptake of less than 1 per cent. After 4 injections of 12.5 units thyrotropic hormone the $I^{131}$ uptake was 2.3 per cent. The vaginal smear was now only 30 per cent cornified and the 17-ketosteroid excretion was 1.7 mg. per 24 hours. An insulin-glucose tolerance test was normal.

It was difficult to conceive of a pituitary tumor of sufficient size to produce papilledema by blockage of the third ventricle without producing peripheral constriction of the visual fields. Furthermore, there was endocrinologic evidence that the abnormal pituitary secretory pattern had been interrupted and that this had been replaced by normal pituitary function. A ventriculogram was performed by the partial replacement of left ventricular fluid with air. The left ventricle was well outlined, although smaller than normal, and the third ventricle was in a normal position. Some air was in the fourth ventricle and basal cisterns. It was concluded that the increased intracranial pressure was on the basis of some physiologic shifts in the fluid compartments and was in some way related either to the child's therapy with triiodothyronine or perhaps to her very rapid growth. In any event, the picture was that of pseudotumor cerebri rather than that of a pituitary adenoma. This syndrome typically occurs in females during a rapidly progressing adolescence.[3] The triiodothyronine was replaced by ½ grain of desiccated thyroid daily and repeated lumbar punctures were carried out to keep the spinal fluid pressure within normal limits to preserve vision. The initial spinal fluid pressure had been over 350 mm. of water, but 3 weeks later the spinal fluid pressure was normal, the hemorrhages in the eye grounds were resorbing, the vision had improved to 20/15, and the papilledema was subsiding. The dose of desiccated thyroid was therefore increased to 1½ grains daily.

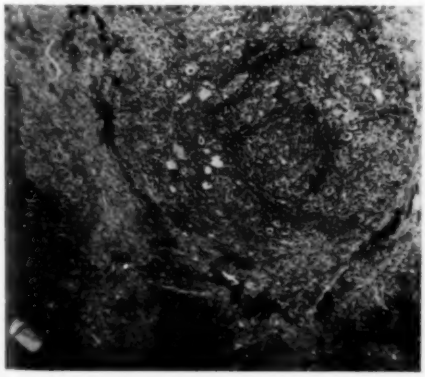


**Fig. 8A.** Sections of thyroid from biopsy (Case 2). The changes are characteristic of Hashimoto's struma with no normal thyroid tissue visible. ×50.

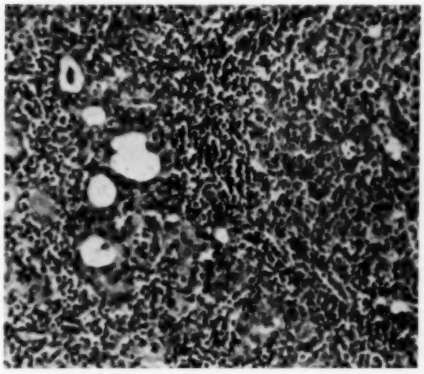


**Fig. 8B.** Thyroid biopsy (Case 2). ×200.

At the age of 13 5/12 years, 8 months after the institution of thyroid therapy, pubic and axillary hair was first noted and at this time she had a normal menstrual period lasting 4 days. She has subsequently continued to menstruate regularly every 28 days with a normal flow.

At 13 8/12 years she began to have daytime polyuria and polydipsia with little or no nocturia. Re-examination of the skull x-rays revealed that the coronal sutures had re-united and the skull was better calcified, but there was further enlargement of the pituitary fossa. A 23-hour urine volume was 2,200 ml. with a specific gravity of 1.001. The 17-ketosteroids were 2.3 mg. per 24 hours, and the 17-hydroxycorticosteroids were 4.2 mg. per 24 hours.

A Hickey-Hare test was carried out in the ambulatory clinic after thirsting overnight.* The 6 A.M. specimen, collected at home, had a concentration of 626 mOsm. per liter. On the way to the hospital the patient became car sick and on arrival at 9:30 A.M. she was slightly dehydrated with a serum osmolarity of 286 mOsm. per liter. With no further hydration she spontaneously began to excrete a hypotonic urine (175 mOsm. per liter). After oral rehydration an intravenous infusion containing 5 per cent saline was administered at the rate of 7.5 ml. per minute. During this infusion the urine osmolarity failed to rise above 79 mOsm. per liter. Substitution of Pitressin (1 mU. per minute) in the intravenous infusion promptly reduced the urine flow and increased the osmolarity of the urine to 470 mOsm. per liter. It would seem from these data that the overnight thirst plus the car sickness had exhausted the limited reserve of antidiuretic hormone to the extent that she was unable to secrete further antidiuretic hormone in response to the stimulus of the Hickey-Hare test. These observations are consistent with the history of daytime polyuria and polydipsia but little or no nocturia. The mild diabetes insipidus and progressive sellar enlargement

*We are indebted to Dr. Robert Winters for carrying out this test.

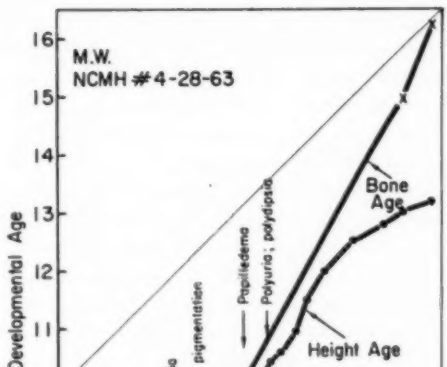



Fig. 9. Growth curve (Case 2).

again suggested the possibility of a chromophobe adenoma, although the visual fields remained normal and rapid growth continued.

In the belief that a pituitary adenoma arising under these circumstances might be inhibited if all stimuli for secretion of TSH were removed; the dosage of desiccated thyroid was further increased to 3 grains daily. Within 6 weeks the excessive thirst and polyuria had disappeared. Subsequent skull x-rays have shown an absolute decrease in the size of the pituitary fossa and better mineralization of the sellar margins. When examined recently at the age of 16 4/12 years the patient was alert and well developed. The pituitary fossa was no longer enlarged (Fig. 7). For the past few months she had experienced cramps in association with otherwise normal menstrual periods. At this time the serum was tested for the presence of thyroid antibodies. These were present in a titer of 1 to 40 by the red cell hemagglutination technique, but

none were detected by the complement fixation or fluorescein-labelled antibody procedure.* The thyroid isthmus was barely palpable. The specific gravity of a 24-hour urine specimen was 1.025. The 17-ketosteroids were 4.9 mg. per 24 hours, the 17-hydroxycorticosteroids were 8.8 mg. per 24 hours, and the urinary FSH excretion was greater than 50 and less than 100 M.U. per 24 hours. Her height was 60.2 inches and her bone age was 16 years (Fig. 9). These findings indicate normal pituitary function and there are no findings to suggest that a pituitary tumor is present.

**Case 3.** (S. D. Babies Hospital 141-14-30). A 7 1/12-year-old girl was referred to the Babies Hospital† because of signs of sexual precocity 6 months prior to admission. The parents were in good health, of average stature, and their pubertal development had been uneventful. The patient's siblings, a younger brother and sister, were in good health. No history of endocrine disorders was obtained. The patient was born 3 weeks before the expected date of birth and weighed 5 pounds, 11 ounces. Growth and development during infancy and early childhood were unremarkable and followed a normal pattern. Early in life the child had been tall for her age. At 1 year of age she had a brief, generalized seizure associated with a high fever and an upper respiratory infection.

The patient was then in good health until about 5 years of age when symptoms of hypothyroidism developed insidiously. She had had some constipation from the age of 3. During the year preceding admission this symptom became more severe and she passed 1 to 2 stools per week. Her rate of growth had sharply decreased during the 2 years preceding admission, and the parents had not noted any increase in stature for the last 6 months. Despite a decrease in appetite the patient began to gain weight at an abnormally rapid rate. She became

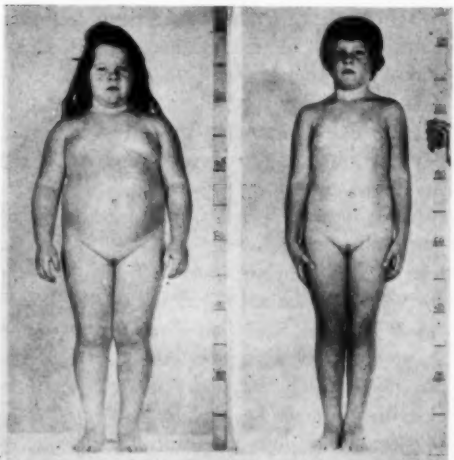



**Fig. 10.** Photographs taken prior to treatment and after 8 months' therapy (Case 3).

sluggish in all her behavior and lacked stamina. Following the loss of her lower central deciduous incisors at 5 years of age, the permanent incisors did not erupt for over 1 year.

Nine months prior to admission, enlargement of the breasts and development of the nipples were noted. Shortly thereafter, a scanty mucoid vaginal discharge was observed. Two months before admission the patient had a 9-day episode of vaginal bleeding which was characterized mainly by spotting. A second episode occurred 3 weeks later and lasted for 5 days; during this time the flow was somewhat heavier, requiring 4 pads daily.

The patient did not have headaches, excessive thirst, nocturia, polyuria, somnolence, or disturbances of gait or vision. She had done well in school, and her mental development was normal.

At the time of admission to the hospital she was pleasant, sluggish, of average height, and seemed more bloated than obese (Fig. 10). Her complexion was sallow and pale; the skin was cool and dry; the hair was brittle and slightly coarse. Bluish mottling

*We are indebted to Dr. Robert Blizzard of Columbus, Ohio, for carrying out the immunologic tests.

†The patient was referred by Dr. Francis De Lorenzo, Montclair, New Jersey, who saw her in consultation.

of the skin was noted over the extremities. She had an impassive, unconcerned expression, and the facies seemed immature for her age. Puffiness of the upper lids was evident. The voice was juvenile but husky. The isthmus of the thyroid gland was easily palpable and thought to be slightly enlarged. The temperature was 98° F., pulse rate 78, and blood pressure 98/80. Her height was 48 inches (height age 7½ years) and weight 87 pounds (average for height is 49.5 pounds). The first 4 permanent molars and the 2 lower permanent central incisors had erupted. Many of the 14 remaining deciduous teeth had caries.

*Sexual development.* Both breasts were enlarged with the glandular tissue on the right measuring about 4 cm. by 6 cm. and on the left 4.5 cm. by 4 cm. The areolae were enlarged, lightly pigmented, and prominent; they measured 3 cm. by 3 cm. on the right, and 2.5 cm. by 2.5 cm. on the left. No secretion could be expressed from either breast at the initial examination; 5 days later, however, about 1 to 2 ml. of a thin, white, opalescent fluid with the appearance of colostrum was readily obtained from each breast. No pubic or axillary hair was present. The labia minora were enlarged and the vaginal mucosa was a dull pink, rugated, thickened, and showed evidence of an estrogenic effect. A white mucoid discharge was present. An adolescent-sized uterus was felt on rectal examination. The clitoris was of normal size. No acne, seborrhea, or hirsutism was observed. There was a moderate growth of soft, brown hair over the back and extremities.

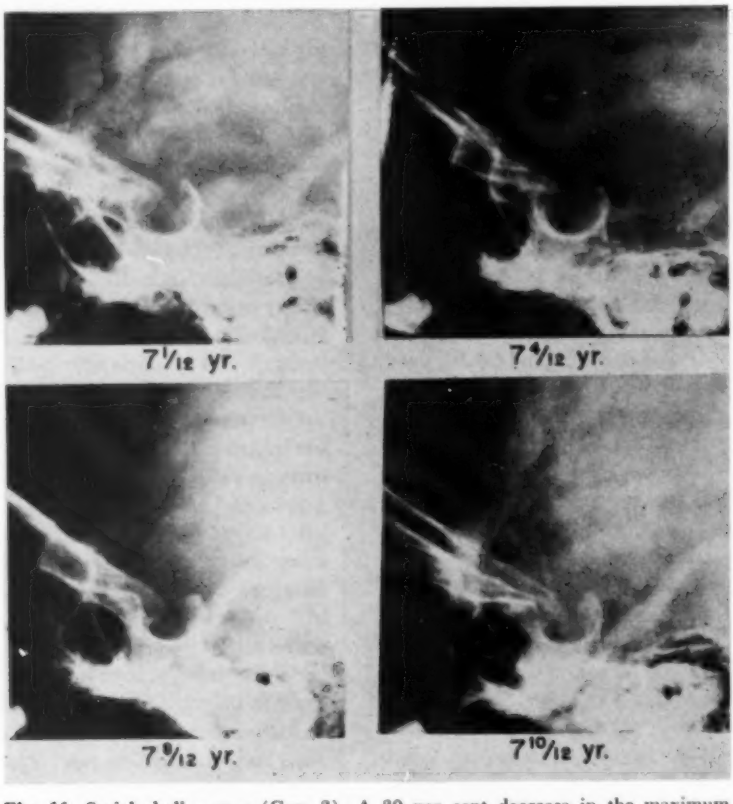



**Fig. 11.** Serial skull x-rays (Case 3). A 30 per cent decrease in the maximum area of the pituitary fossa has occurred between the first and last films.

Visual acuity was 20/25 for the left eye and 20/30 for the right eye. The fundi and the visual fields examined by confrontation perimetry were normal. No neurological abnormalities were detected; there was no delay in the relaxation of the deep tendon reflexes. A detailed neurological examination by Dr. Sidney Carter revealed no abnormality.

Gynecological examination by Dr. Anna Southam under anesthesia revealed a uterus of average size for an adolescent. Both ovaries were palpable, almond shaped, and noncystic, with dimensions of about 2 by 2.5 cm. A biopsy of the endometrium showed glands in a proliferative phase. Inflammatory cells and blood were seen within the stroma. These findings were compatible with menstruation.

The ECG showed depression of the T waves and slowed conduction rates. X-ray examination of the wrist was interpreted as consistent with a bone age of $5\frac{9}{12}$ years; no signs of epiphysial dysgenesis were detected. An x-ray film of the skull showed an enlarged pituitary fossa in the lateral view (Fig. 11).* The maximum anteroposterior diameter was 16 mm., and the minimum was 13 mm. The area of the sella turcica as determined by the method of Hare[2] was 150 mm. The dorsum sellae was unusually thin and demineralized. Lumbar puncture revealed clear, colorless fluid with an initial pressure of 175 mm. of water. The protein content was 32 mg. per 100 ml. and sugar 59 mg. per 100 ml. Multiple projections of the skull obtained after the replacement of 45 ml. of cerebrospinal fluid by air, demonstrated excellent filling of the ventricular system and basal cisterns. These films were interpreted by Dr. Juan Taveras as demonstrating a small suprasellar mass impinging on the cisterna chiasmatica. The EEG was normal. Visual fields by tangent screen revealed no constriction in field perimeters.

*We are grateful to Dr. Juan Taveras and Dr. John Caffey of the Department of Radiology, Columbia-Presbyterian Medical Center, for the measurements of the area of the pituitary fossa.

The hemogram was normal. Examination of a specimen of urine showed a specific gravity of 1.023, pH 5.5, a trace of albumin, sugar 0, acetone 0, and 10 to 15 white blood cells per high-power field. The serum calcium concentration was 11.7 mg. per 100 ml., phosphorus 4.1 mg. per 100 ml., alkaline phosphatase 6.0 Bodansky units, and cholesterol 277 mg. per 100 ml. The zinc turbidity of the serum was elevated to 19.8 units. The total serum protein content was 8.4 Gm. per 100 ml. The concentration of gamma globulin estimated following electrophoresis of serum on paper was 0.93 Gm. per 100 ml.

The serum protein bound iodine content was 2.4 $\mu$g./100 ml. both before administration of TSH and after 5 units of TSH every 12 hours for 6 doses. The thyroidal uptake of $I^{131}$ was 7 per cent of the administered dose at 4 hours and 15 per cent at 24 hours. After TSH stimulation there was no significant change. A water loading test was normal with 92 per cent of the administered dose being excreted in 3 hours. The urinary excretion of 17-ketosteroids was 2.0 to 2.4 mg. per 24 hours. The concentration of free plasma 17-hydroxycorticosteroids was 8.7 $\mu$g. per 100 ml. and increased to 39.3 $\mu$g. per 100 ml. after stimulation with 25 units ACTH administered intravenously over a 4 hour period. The biological half-time of plasma hydrocortisone was prolonged to 135 minutes. After treatment with triidothyronine for 2 weeks the half time decreased to 95 minutes, a normal value. Urinary gonadotropins on two occasions were less than 5 M.U. per 24 hours. The vaginal smear contained many cornified cells and large bluish cells with small pyknotic nuclei, indicating a full estrogenic effect. The serum exhibited no thyroid antibodies by the red cell hemagglutination or complement fixation techniques, but gave a two plus reaction in the Coons fluorescein-labeled antibody procedure.*

*We are indebted to Dr. Robert Blizzard of Columbus, Ohio, for carrying out the immunologic tests.

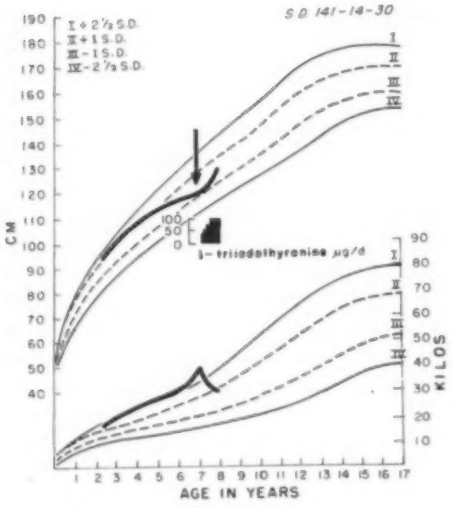



**Fig. 12.** Growth curve (Case 3).

*Course.* Several days after the patient was admitted to the hospital she had another menstrual period which lasted for 5 days. In addition, spontaneous galactorrhea was noted and this persisted throughout the remainder of her admission. This consisted of the secretion of a few drops of a thin, milky fluid from each breast. Microscopic examination of this fluid obtained by manual expression and stained with Sudan III showed numerous fat globules.

Treatment with l-triiodothyronine was instituted 2 weeks after admission. The dose was gradually increased over a 10-day period from 37.5 Mcg. daily by mouth to 50 Mcg. twice a day. The response to this therapy was dramatic, and, when examined 6 weeks after triiodothyronine treatment, the child was euthyroid, much more alert, and no longer constipated. During this period she lost 8.5 pounds in weight. Her hair had become less coarse. The skin was softer and for the first time in several years her lips and cheeks were rosy in color. The pulse rate had increased to 116 per minute and the blood pressure was now 120/60. The galactorrhea had ceased and no further menses had occurred.

Ten weeks after discharge the patient began to limp and complained of occasional pain in her right hip. Roentgenograms of the hips showed a slipped capital femoral epiphysis and the characteristic destructive changes in the metaphysis associated with this condition. A McLaughlin nail was inserted through the lateral cortex of the femur into the femoral neck and the capital femoral epiphysis by Dr. Charles Ryder. Recovery was uneventful and the child was walking unassisted without discomfort 3 months later.

When the patient was seen at $7\frac{10}{12}$ years, 8 months after the institution of therapy, she had grown 2.8 inches in 8 months and had lost 18 pounds (Fig. 12). Five permanent incisors had erupted. An x-ray film of the wrist showed a bone age of 8 years. The puffiness of the face had disappeared and her facies was more mature. The breast tissue had diminished considerably in size and the breasts were now mainly composed of fatty tissue. The areolae no longer showed evidence of stimulation by estrogen and were not pigmented. The isthmus of the thyroid gland was not palpable. No pubic or axillary hair was present and much of the long, soft hair over her back and extremities had disappeared. The labia minora were prepubescent in appearance; the vaginal mucosa was pink and glistening, and no mucoid vaginal discharge was detected. A smear of the vaginal mucosa contained cells characteristic of the preadolescent period. No cornified cells were seen; most of the cells were intermediate in size, stained bluish green, and contained large, vesicular nuclei. A moderate number of polymorphonuclear leukocytes were present. The concentration of serum protein-bound iodine was 0.9 μg. per 100 ml. The patient was last examined at $8\frac{5}{12}$ years, 15 months after treatment was begun. No signs of precocious sexual development were detected. She measured 53 inches in height, an increment of 5 inches since the institution of therapy. Triiodothyronine was discontinued and replaced with desiccated thyroid, 128 mg. daily.

During the entire period of observation,

repeated examinations of the fundi and of the visual fields by tangent screen were normal. No abnormal neurological signs were noted. Roentgen examinations of the pituitary fossa showed a gradual reduction in size. In 8 months the dorsum sellae had remineralized, the maximum anteroposterior diameter was 13 mm., and the maximum area was 100 sq. mm., a reduction of 30 per cent. A clear-cut decrease in the size of the pituitary fossa is apparent in Fig. 11. The double contour line observed in the film taken earlier is no longer evident.

## DISCUSSION

**Definition of syndrome and review of the literature.** In each of these 3 patients the hypothyroidism was due to primary failure of the thyroid gland and not secondary to pituitary insufficiency. This was demonstrated in each instance by the failure to respond significantly to TSH. In 1 (Case 2) the diagnosis of Hashimoto's struma, established initially by biopsy of the thyroid gland, was supported by the presence of circulating thyroidal antibodies 3 years later. In another (Case 3) the increased zinc turbidity of the serum and the presence of thyroidal antibodies suggested a similar pathogenesis although a biopsy of the thyroid was not obtained.

In addition to primary hypothyroidism each of the 3 patients had precocious menstruation, enlargement of the breasts, and galactorrhea, although pubic hair was not evident and the bone ages were retarded. Increased pigmentation was present in 1 patient. The pituitary fossa was enlarged in all 3. All of these abnormal findings were reversed by the administration of thyroid hormone.

At least 3 additional girls and 1 boy with hypothyroidism with various manifestations of sexual precocity have been previously described,[4-7] although lactation and pigmentation were not mentioned in these reports. In 1905 Kendle[4] described an inadequately treated cretin of the familial type who developed irregular menses at 5 7/12 years. At 9 years she had fully developed breasts with prominent nipples and coarse axillary and pubic hair. Kendle's observations 1 year after the resumption of treatment with thyroid extract are noteworthy: "Strange to say she has not menstruated since she resumed the thyroid extract: the breasts are much smaller, the nipples less prominent; hair has disappeared from the axillae and pubis; the voice is less deep, and her whole organism seems to have reverted to a more childlike type. This is the more astonishing, since it is a well known fact that puberty is invariably delayed in cretins and that treatment tends to ripen the uterine functions in those cases in which puberty has not run paripassu with adolescence."

Similar observations were made following the administration of thyroid to the girls described by Bergstrand[5] and Silver.[6] Both patients had precocious menstruation and enlargement of the breasts, although neither had pubic hair before treatment with thyroid was instituted. Bergstrand's patient had an enlarged sella turcica, and, like the patients described in this paper, she underwent a normal menarche at the age of 15, 6 years after the institution of therapy with thyroid extract. The boy described by Hubble[7] was a 25-year-old untreated cretin with a height of 46 inches and a bone age of 11 to 12 years. Although he lacked pubic hair, his penis, scrotum, and testes were larger than those of a normal adult male. With the institution of thyroidal therapy the sexual organs became smaller and pubic hair appeared.

The association of hypothyroidism with various manifestations of sexual precocity and the reversion to a prepubescent state following the institution of therapy with thyroid appear to be the essential findings in this syndrome. Galactorrhea, pigmentation, and enlargement of the pituitary fossa are additional findings in some cases. These distinctive features in a child with retarded skeletal age should serve to distinguish this syndrome from the ordinary type of precocious puberty in which the normal hypo-

thalamic-pituitary mechanism is activated prematurely.

**Relationship to chromophobe adenomas of the pituitary.** Enlargement of the pituitary and sella turcica in long standing myxedema was recognized over a century ago[8] and has been confirmed many times since.[9, 12] It is generally assumed that the pituitary enlargement stems from hyperplasia of the cells concerned with the compensatory secretion of thyrotropic hormone. Histologic studies of the pituitary glands in such patients reveal hyperplasia of cells known as "amphophil cells" in the terminology of Russfield[12] or "vesiculated gamma cells" in the classification of Ezrin.[13] These cells are considered to be indicative of an active secretory state, although by conventional stains they would be classified as chromophobes. Assays of the serum reveal elevated levels of TSH in a majority of myxedematous patients.[14, 15]

It is possible that both the changes in the pituitary fossa and the endocrine abnormalities of the patients described in this paper were attributable to chromophobe adenomas rather than to hyperplasia of the pituitary as described above. The suprasellar mass demonstrated by pneumoencephalography in 1 (Case 3) (the only patient so studied), the mild diabetes insipidus of another (Case 2), and the rarefaction and enlargement of the sella turcica demonstrated in all 3 are characteristic of chromophobe adenomas. The absence of visual field defects suggests that the adenomas, if present, were relatively small. In a series of 117 patients with classical chromophobe adenomas reported from the Neurological Institute in New York City, impairment of the visual fields was present in 96 per cent of them at the time when they were first seen.[16] This incidence may be unduly high since patients with smaller tumors and lacking obvious neurological signs would be less likely to be referred to that center.

The fact that the endocrine abnormalities and enlargement of the pituitary fossae were reversed when thyroid was administered further suggests that these pathologic changes were due to hyperplasia rather than adenoma. It should be recalled, however, that suppressibility of hormonal secretion through the feedback mechanism does not always distinguish between adenoma and hyperplasia of the thyroid and adrenal glands. There is no a priori reason to believe that pituitary adenomas behave differently from those arising in other endocrine glands. It would appear that the distinction between compensatory hyperplasia and chromophobe adenoma of the pituitary is a nebulous one and that at least some chromophobe adenomas develop in response to pituitary hyperplasia arising from some recognized or unrecognized target gland deficiency. This concept, clearly expressed by Russfield,[17] is supported by evidence obtained in studies of experimental tumors.[18]

Pituitary tumors have been produced in susceptible laboratory animals rendered hypothyroid by thyroid ablation,[19] by administration of $I^{131}$,[20] and by prolonged feeding of propylthiouracil.[21] Other pituitary tumors have been produced by adrenalectomy, castration, or by administration of estrogen. For a time these metastasizing adenomas regress when the target gland deficiency of the host is alleviated or when administration of estrogen is discontinued. As the tumors become less differentiated, their secretion of tropic hormones diminishes and they no longer regress when adequate target gland substitution therapy is provided.[18]

Instances of pituitary tumors developing in man after a period of primary target gland deficiency have likewise been documented. One such tumor arising in a woman with long-standing hypothyroidism gave rise to galactorrhea and menorrhagia.[22] A number of patients from various clinics have developed pituitary tumors that secreted ACTH following adrenalectomy for Cushing's syndrome.[23, 24] One such patient, seen by one of the authors, presented with galactorrhea as well as increased pigmentation. Hypophyseal tumors have been reported in the Skopec tribes following ritual castration.[25]

Symptoms of sexual dysfunction often antedate the recognition of chromophobe adenomas by many years; in one series the average duration was 12 years.[26] Is it possible that some of these pituitary adenomas developed in response to a primary hypogonadal state? The assumption that the hypogonadism frequently encountered in patients with pituitary tumors is secondary to pituitary insufficiency is based on the frequent finding that the excretion of gonadotropic hormone is either diminished or absent at the time that the tumor is recognized. The finding of low gonadotropic hormone excretion at this time may simply reflect secondary pituitary insufficiency brought about by the replacement of functional pituitary tissue by less differentiated tumor cells. Such a sequence leading to secondary atrophy of all elements stimulated by the pituitary body occurs when experimental pituitary tumors become large.[25]

**Hormonal overlap in the pituitary feedback mechanism.** Hypothyroidism is known to produce severe disturbances in the menstrual patterns of older cretins who are undertreated and in women who have acquired primary myxedema. The clinical picture is usually that of ovulatory failure and of metropathic hemorrhagica due to sustained hyperestrinism.[28] The extensive literature bearing on the exact mechanism of these changes, however, is confusing and inconclusive.[29] It has been adequately demonstrated that the hypothyroid animal is excessively sensitive to small amounts of exogenous gonadotropin.[30, 31, 32] The hypothyroid patient also has a prolonged turnover rate of steroid hormones, as demonstrated by the slow rate of disappearance of hydrocortisone in our patient (Case 3). Delayed metabolism of or hypersensitivity to gonadotropic hormones or estrogens may play an important role in the menorrhagia of mature hypothyroid women. This mechanism is more difficult to accept as a satisfactory explanation for the sexual development of prepubescent girls, however, since their endogenous secretion of gonadotropins and estrogens is ordinarily very slight. A more reasonable interpretation of the findings manifested in these prepubescent girls is that the hyperplastic pituitary, in the process of secreting large quantities of thyrotropic hormone, also secreted gonadotropins, mammotropic hormone, and in 1 (Case 2), melanocyte-stimulating hormone.

The concept that the pituitary may respond to a single target gland deficiency with oversecretion of multiple heterologous hormones finds support in the experimentally induced pituitary tumors as well as in many clinical observations. Animals developing pituitary tumors in response to hypothyroidism exhibit marked ovarian stimulation as well as cystic hyperplasia of the endometrium and breasts.[18, 33] The gonadotropic activity of these tumors appears to be quantitatively related to the thyrotropic activity. In all probability the mammotropic activity does not regularly accompany TSH, but rather is due to secondary secretion of mammotropin in response to the high estrogen levels in these animals.[18] ACTH-secreting tumors regularly have a melanocyte-stimulating activity and occasionally mammotropic activity as well. In experimentally induced mammotropic tumors, the somatotropic effect is proportional to the effect on the breasts.[18]

Numerous clinical observations likewise suggest that the pituitary feedback is less specific than might be inferred from the reciprocal arrows which are commonly drawn to illustrate hypophyseal-target gland relationships. Galactorrhea, a symptom commonly present in pituitary dysfunction of various sorts, has been described in association with both hypothyroidism and hyperthyroidism.[22, 34, 35] Hyperplasia of both the endometrium and mammary glands is present in a high percentage of women dying with primary thyroid disorders.[12] The presence of these changes in the uterus and breasts has a high degree of correlation with the percentage of amphophil cells in the pituitary.

Multihormonal secretory patterns have likewise been described in castrates and in patients with primary gonadal insufficiency.

In patients with Klinefelter's syndrome there is an increased incidence of thyroid and adrenal adenomas and 1 such patient had hyperplasia of the amphophil cells in his pituitary.[36] The gynecomastia which many of these patients develop may therefore be due to the secretion of heterologous hormones by the pituitary along with the gonadotropic hormone secreted in response to the primary hypogonadism. The somatic overgrowth of eunuchs, in part caused by delayed epiphyseal fusion, might similarly in part be explained by increased elaboration of somatotropin.

Castrated animals[37] and patients with primary hypogonadism[38] have been reported to secrete excessive amounts of thyroid-stimulating hormone. Asper[39] has reported 6 patients with long-standing primary hypogonadism who developed Graves' disease. One of von Basedow's[40] original patients with hyperthyroidism had gynecomastia and lactation. Hyperthyroidism not infrequently follows Addison's disease.[41] A subnormal amount of axillary hair was observed in 50 of 102 patients with Graves' disease, and in many cases the deficiency was of long standing.[42] Such observations serve to support the hypothesis of Marine[43] that Graves' disease may stem from a compensatory reaction to primary abnormalities in the adrenals or gonads.

Although the main features of acromegaly are related to oversecretion of growth hormone, thyroid and adrenal enlargement is common. One of us has recently studied a 27-year-old man with acromegaly who had a breast tumor composed of actively proliferating duct tissue. A similar patient with lactation has been described.[44] The gynecomastia which is typically seen in boys with rapidly progressing adolescence might likewise be accounted for on the basis of overlapping pituitary secretions. There is an increased association between gynecomastia and thyroid enlargement in otherwise normal boys who are rapidly passing through adolescence.[45]

The frequency with which an overlap in pituitary secretions may be inferred to occur in response to a single target gland deficiency suggests that the administration of a single target gland hormone may inhibit more than its specific tropic hormone. The administration of thyroid to the patients described in this paper led to the inhibition of the abnormal menses, lactation, and the hyperpigmentation at a time when suppression of TSH presumably occurred. The administration of estrogen is said to inhibit certain manifestations of acromegaly[46] and Cushing's syndrome.[47] Stilbestrol and cortisone are effective in decreasing secretion of TSH in rabbits in a manner that cannot be accounted for on the basis of the usual thyroid-TSH feedback.[48] These observations lend further support to the concept that an alteration in the secretory status of one tropic hormone is sometimes associated with secondary alterations in the secretion of other tropic hormones as well.

**Locus of hormonal overlap.** The locus of at least some of this hormonal overlap is at the molecular level. It has been amply demonstrated that melanocyte-stimulating activity is inherent in the ACTH molecule.[49] An overlap in function between synthetic oxytocin and vasopressin has also been well documented.[50] Since TSH and the gonadotropins are glycoproteins it is possible that a similar molecular overlap exists between these hormones. Somatotropin and mammotropin likewise have certain chemical similarities and their biological effects are usually associated in experimental tumors.

If the multihormonal responses of the pituitary are simply the result of overlapping molecular structures, there should be a regular and predictable relationship between the secretion of one hormone and the appearance of another type of hormonal activity. The situation appears to be more complex than this, however, and it is difficult to explain the variability in hormonal patterns encountered in experimental pituitary tumors and in various clinical endocrinopathies on this basis. It would be difficult, for example, to explain the hyperpigmentation of 1 (Case 2) on the basis of a chemical overlap between thyroid-stimu-

lating hormone and melanocyte-stimulating hormone, although this possibility cannot be excluded. The overlap here might be at the cellular level with more than one cell type undergoing hyperplasia, or a specific cell type may be concerned with the synthesis of multiple hormones.[12, 13]

Another possible site where hormonal overlap might occur is at the hypothalamic level. Since the pituitary feedback is in part mediated through the hypothalamic nuclei, the release of multiple heterologous pituitary hormones might result equally well from lack of specificity in the hypothalamic mechanisms which mediate their synthesis and release.

Until the molecular structure of the hypothalamic and hypophyseal hormones is known and more is understood about the factors which govern their synthesis and release, observations on pituitary overlap must necessarily be empirical and inferential. Pigmentation of the patient with Addison's disease was recognized long before knowledge of the overlapping chemical structures of ACTH and MSH provided a complete explanation of this phenomenon. Most pituitary hormones do not declare their presence as unequivocally as does MSH and the currently available biological assays are at best crude and unreliable indices of the secretory status of the pituitary. Nevertheless, since we have become attentive to the possibility of pituitary overlap, it has been impressive how many well-known and in some instances paradoxical observations in clinical endocrinology may be explained readily by this concept.

## SUMMARY

A syndrome associated with juvenile hypothyroidism is described, the cardinal feature of which is sexual development beyond that consistent with the bone age and other indices of maturity. The 3 girls described in this paper had precocious menstruation, galactorrhea, absence of pubic hair, and enlargement of the sella turcica. One patient was excessively pigmented. These abnormal signs disappeared promptly when the hypothyroid state was alleviated. Serial x-rays of the skull after treatment with thyroid was instituted revealed an initial period of further enlargement and demineralization of the pituitary fossa followed by a decrease in its size toward normal. A normal menarche later ensued in 2 of the girls when the level of maturity had progressed appropriately. The implications of the close similarity between the findings in this syndrome and in patients with pituitary tumors are discussed. A primary target gland deficiency is suggested as the etiologic factor in at least some patients with chromophobe adenomas.

It is postulated that the mechanism for the development of menorrhagia, galactorrhea, and pigmentation in these patients was an overlapping secretion of gonadotropin, mammotropic hormone, and (in 1 case) melanocyte-stimulating hormone along with the presumed high level of thyroid-stimulating hormone. A similar overlap in pituitary hormones occurs in experimental pituitary tumors induced by single target gland deficiencies and in certain clinical endocrine disorders. This lack of specificity in the pituitary feedback mechanism, so strikingly demonstrated in the present syndrome, is suggested as the mechanism responsible for a number of phenomena commonly observed in clinical endocrine disorders.

## REFERENCES

1. Silverman, F. N.: Roentgen Standards for Size of the Pituitary Fossa From Infancy Through Adolescence, Am. J. Roentgenol. **78:** 451, 1957.
2. Hare, H. F., Silveus, E., Smedal, M. I.: Roentgenologic Diagnosis of Pituitary Tumors, Endocrinology **52:** 193, 1949.
3. Walsh, F. B.: Personal communication.
4. Kendle, F. W.: Case of Precocious Puberty in a Female Cretin, Brit. M. J. **1:** 246, 1905.
5. Bergstrand, C. G.: A Case of Hypothyroidism With Signs of Precocious Sexual Development, Acta endocrinol. **20:** 338, 1955.
6. Silver, H. K.: Juvenile Hypothyroidism With Precocious Sexual Development, J. Clin. Endocrinol. **18:** 886, 1958.
7. Hubble, D.: Endocrine Relations, Lancet **1:** 1, 1955.

8. Niépce, B.: Traité du goitre et du cretinisme, Paris, 1851, J. B. Bailliere.
9. Boyce, R., and Beadles, C. F.: Enlargement of the Hypophysis Cerebri in Myxedema; With Remarks Upon Hypertrophy of the Hypophyses, Associated With Changes in the Thyroid Body, J. Path. & Bact. **1:** 223, 1892.
10. Pende, V.: Sopra un segno cranio-radiologico frequente nel mixedema, Arch. "E. Maragliano" pat e clin. **8:** 616, 1953.
11. Beltinc, M. A., and Neves, I.: The Skull in Childhood Myxedema: Its Roentgen Appearance, Am. J. Roentgenol. **76:** 495, 1956.
12. Russfield, A. B.: Histology of the Human Hypophysis in Thyroid Disease, J. Clin. Endocrinol. **15:** 1393, 1956.
13. Ezrin, C.: The Cells of the Human Adenohypophysis in Thyroid Disorders, J. Clin. Endocrinol. **19:** 958, 1959.
14. Gilliland, I. C., and Strudwick, J. I.: Clinical Application of an Assay of Thyroid-Stimulating Hormone in Relation to Exophthalmus, Brit. M. J. **1:** 378, 1956.
15. D'Angelo, S. A.: Pituitary Regulation of Thyroid Gland Function, in the Thyroid, Brookhaven Symposia in Biology, No. 7, 1955, p. 9.
16. Nurnberger, J. I., and Korey, S. R.: Pituitary Chromophobe Adenomas, a Clinical Study of the Sellar Syndrome, New York, 1953, Springer Pub. Co., Inc., p. 127.
17. Russfield, A. B., Reiner, L., and Klaus, H.: The Endocrine Significance of Hypophyseal Tumors in Man, Am. J. Path. **32:** 1055, 1956.
18. Furth, J., and Clifton, K. H.: Experimental Pituitary Tumors, in Wolstenholme, G. E. W., and O'Connor, M., editors: Ciba Foundation Colloquia on Endocrinology, vol. 12, Hormone Production in Endocrine Tumors, Boston, 1958, Little, Brown & Company, p. 3.
19. Furth, J. Dent, J. N., Burnett, W. T., and Gadsden, E. L.: The Mechanism of Induction and the Characteristics of Pituitary Tumors Induced by Thyroidectomy, J. Clin. Endocrinol. **15:** 81, 1955.
20. Gorbman, A.: Tumorous Growths in the Pituitary and Trachea Following Radiotoxic Dosages of $I^{131}$, Proc. Soc. Exper. Biol. & Med. **71:** 237, 1949.
21. Moore, G. E., Brackney, E. L., and Bock, F. G.: Production of Pituitary Tumors in Mice by Chronic Administration of a Thiouracil Derivative, Proc. Soc. Exper. Biol. & Med. **82:** 643, 1953.
22. Salus, F.: Über einen Fall van Hypophysengangsgeschwulst mit Galaktorrhöe, Deutsches Arch. f. klin. Med. **177:** 614, 1935.
23. Salassa, R. M., Kearns, T. P., Kernohan, J. W., Sprague, R. G., and MacCarty, C. S.: Pituitary Tumors in Patients With Cushing's Syndrome, J. Clin. Endocrinol. **19:** 1523, 1959.
24. Montgomery, D. A. D., Welbourn, R. B., McGaughey, W. T. E., and Gleadhill, C. A.: Pituitary Tumours Manifested After Adrenalectomy for Cushing's Syndrome, Lancet **2:** 707, 1959.
25. Tandler, J., and Gross, S.: Über den Einfluss der Kastration aufden Organismus. II. Die Skopzen, Arch. Entwcklngsmechn. Organ. **30:** 2364, 1910.
26. Nurnberger, J. I., and Korey, S. R.: Pituitary Chromophobe Adenomas, a Clinical Study of the Sellar Syndrome, New York, 1953, Springer Pub. Co., Inc., p. 122.
27. Furth, J.: Morphological Changes Associated With Thyrotropin-Secreting Pituitary Tumors, Am. J. Path. **30:** 421, 1954.
28. Goldsmith, R. E., Somers, H. S., Lerman, J., and Stanbury, J. B.: The Menstrual Pattern in Thyroid Disease, J. Clin. Endocrinol. **12:** 846, 1952.
29. Rogers, J.: Medical Progress, "Menstruation and Systemic Disease," Thyroid Disorders, New England J. Med. **259:** 676, 721, and 770, 1958.
30. Janes, R. G.: Effect of a Pituitary Gonadotrophin on the Ovaries of Hypothyroid Rats, Endocrinology **54:** 464, 1954.
31. Chu, J. P.: Influence of the Thyroid Gland on Pituitary Gonadotrophin Activity in the Rabbit, Endocrinology **34:** 90, 1944.
32. Mandl, A. M.: Factors Influencing Ovarian Sensitivity to Gonadotrophins, J. Endocrinol. **15:** 448, 1957.
33. Sommers, S. C., Chute, R. N., and Burt, A. S.: Cystic Hyperplasia of Endometrium and Breast in Mice With $I^{131}$ Induced Pituitary Adenomas (Abst.), Am. J. Path. **30:** 621, 1954.
34. Jackson, W. P. U.: Post-Thyroidectomy Hypothyroidism, Hypoparathyroidism, Exophthalmos and Galactorrhea With Normal Menstruation: Metabolic Response to Probenecid, J. Clin. Endocrinol. **16:** 1245, 1956.
35. Zondek, B., Bromberg, Y. M., and Rozin, S.: An Anterior Pituitary Hyperhormonotrophic Syndrome, J. Obst. & Gynaec. Brit. Emp. **58:** 525, 1951.
36. Burt, A. S., Reiner, L., Cohen, R. B., and Sniffen, R. C.: Klinefelter's Syndrome: Report of Autopsy With Particular Reference to Histology and Histochemistry of the Endocrine Glands, J. Clin. Endocrinol. **14:** 719, 1954.
37. Aron, M., and Benoit, J.: Influence de la castration sur le taux d'hormone préhypophysaire excito-sécrétrice de la thyroïde present dans le milieu intérieur, chez le cobaye, Compt. rend. Soc. de Biol. **83:** 734, 1931.
38. Fromm, G. A., Bur, E. B., Del Conte, E., Lascano, E. F., and Hecker, E.: Prepubertal Gonadal Insufficiency: Its Correlation With the Adrenals and Blood Thyrotropin, Acta endocrinol. **19:** 112, 1955.
39. Asper, S. P., and Wilson, E. H.: Coexisting Testicular Deficiency and Hyperthyroidism—By Design or By Chance, Tr. A. Am. Physicians **70:** 148, 1957.

40. von Basedow, C. A.: Die Glotzangen, Wchnschr. Gesammte Heilkunde **14:** No. 49, 769, 1848.
41. Rupp, J. J., and Paschkis, K. E.: Co-existence of Addison's Disease and Thyrotoxicosis, J. Clin. Endocrinol. **17:** 143, 1957.
42. Williams, R. H.: Thyroid and Adrenal Interrelations With Special Reference to Hypotrichosis Axillaris in Thyrotoxicosis, J. Clin. Endocrinol. **7:** 52, 1947.
43. Marine, D.: Remarks on Pathogenesis of Graves' Disease, Am. J. M. Sc. **180:** 767, 1930.
44. Roth, O.: Auftreten von Milchsekretion bei einem an akromegalie leidenden Patienten, Klin. Wchnschr. **55:** 305, 1918.
45. Rawson, R.: Personal communication.
46. McCullagh, E. P., Beck, J. C., and Schaffenburg, C. A.: Control of Diabetes and Other Features of Acromegaly Following Treatment With Estrogens, Diabetes **4:** 13, 1955.
47. Kovačić, N.: An Attempt to Inhibit Synthesis of Corticosteroid Hormones by Means of Diethylstilboestrol in a Case of Cushing's Syndrome. Preliminary Report, Acta med. scandinav. **164:** (Fasc. 6) 455, 1959.
48. Brown-Grant, K.: The "Feed-Back" Hypothesis of the Control of Thyroid Function, in Wolstenholme, G. E. W., and Millar, E. C. P., editors: The Ciba Foundation Colloquia on Endocrinology, vol. 10, Regulation and Mode of Action of Thyroid Hormones, Boston, 1957, Little, Brown & Company, p. 97.
49. Li, C. H.: Anterior Pituitary Gland Hormones (Part II), in Anson, M. L., Bailey, K., and Edsall, J. C. T., editors: Advances in Protein Chemistry, New York, 1955, Academic Press, Inc., p. 4.
50. Van Dyke, H. B., Adamsons, K., Jr., and Engel, S. L.: Aspects of the Biochemistry and Physiology of the Neurohypophyseal Hormones, in Pincus, Gregory: Recent Progress in Hormone Research, vol. 11, New York City, 1955, Academic Press, Inc., p. 4.

8. Niépce, B.: Traité du goitre et du cretinisme, Paris, 1851, J. B. Bailliere.
9. Boyce, R., and Beadles, C. F.: Enlargement of the Hypophysis Cerebri in Myxedema; With Remarks Upon Hypertrophy of the Hypophyses, Associated With Changes in the Thyroid Body, J. Path. & Bact. **1:** 223, 1892.
10. Pende, V.: Sopra un segno cranio-radiologico frequente nel mixedema, Arch. "E. Maragliano" pat e clin. **8:** 616, 1953.
11. Beltinc, M. A., and Neves, I.: The Skull in Childhood Myxedema: Its Roentgen Appearance, Am. J. Roentgenol. **76:** 495, 1956.
12. Russfield, A. B.: Histology of the Human Hypophysis in Thyroid Disease, J. Clin. Endocrinol. **15:** 1393, 1956.
13. Ezrin, C.: The Cells of the Human Adenohypophysis in Thyroid Disorders, J. Clin. Endocrinol. **19:** 958, 1959.
14. Gilliland, I. C., and Strudwick, J. I.: Clinical Application of an Assay of Thyroid-Stimulating Hormone in Relation to Exophthalmus, Brit. M. J. **1:** 378, 1956.
15. D'Angelo, S. A.: Pituitary Regulation of Thyroid Gland Function, in the Thyroid, Brookhaven Symposia in Biology, No. 7, 1955, p. 9.
16. Nurnberger, J. I., and Korey, S. R.: Pituitary Chromophobe Adenomas, a Clinical Study of the Sellar Syndrome, New York, 1953, Springer Pub. Co., Inc., p. 127.
17. Russfield, A. B., Reiner, L., and Klaus, H.: The Endocrine Significance of Hypophyseal Tumors in Man, Am. J. Path. **32:** 1055, 1956.
18. Furth, J., and Clifton, K. H.: Experimental Pituitary Tumors, in Wolstenholme, G. E. W., and O'Connor, M., editors: Ciba Foundation Colloquia on Endocrinology, vol. 12, Hormone Production in Endocrine Tumors, Boston, 1958, Little, Brown & Company, p. 3.
19. Furth, J. Dent, J. N., Burnett, W. T., and Gadsden, E. L.: The Mechanism of Induction and the Characteristics of Pituitary Tumors Induced by Thyroidectomy, J. Clin. Endocrinol. **15:** 81, 1955.
20. Gorbman, A.: Tumorous Growths in the Pituitary and Trachea Following Radiotoxic Dosages of $I^{131}$, Proc. Soc. Exper. Biol. & Med. **71:** 237, 1949.
21. Moore, G. E., Brackney, E. L., and Bock, F. G.: Production of Pituitary Tumors in Mice by Chronic Administration of a Thiouracil Derivative, Proc. Soc. Exper. Biol. & Med. **82:** 643, 1953.
22. Salus, F.: Über einen Fall van Hypophysengangsgeschwulst mit Galaktorrhöe, Deutsches Arch. f. klin. Med. **177:** 614, 1935.
23. Salassa, R. M., Kearns, T. P., Kernohan, J. W., Sprague, R. G., and MacCarty, C. S.: Pituitary Tumors in Patients With Cushing's Syndrome, J. Clin. Endocrinol. **19:** 1523, 1959.
24. Montgomery, D. A. D., Welbourn, R. B., McGaughey, W. T. E., and Gleadhill, C. A.: Pituitary Tumours Manifested After Adrenalectomy for Cushing's Syndrome, Lancet **2:** 707, 1959.
25. Tandler, J., and Gross, S.: Über den Einfluss der Kastration aufden Organismus. II. Die Skopzen, Arch. Entwcklngsmechn. Organ. **30:** 2364, 1910.
26. Nurnberger, J. I., and Korey, S. R.: Pituitary Chromophobe Adenomas, a Clinical Study of the Sellar Syndrome, New York, 1953, Springer Pub. Co., Inc., p. 122.
27. Furth, J.: Morphological Changes Associated With Thyrotropin-Secreting Pituitary Tumors, Am. J. Path. **30:** 421, 1954.
28. Goldsmith, R. E., Somers, H. S., Lerman, J., and Stanbury, J. B.: The Menstrual Pattern in Thyroid Disease, J. Clin. Endocrinol. **12:** 846, 1952.
29. Rogers, J.: Medical Progress, "Menstruation and Systemic Disease," Thyroid Disorders, New England J. Med. **259:** 676, 721, and 770, 1958.
30. Janes, R. G.: Effect of a Pituitary Gonadotrophin on the Ovaries of Hypothyroid Rats, Endocrinology **54:** 464, 1954.
31. Chu, J. P.: Influence of the Thyroid Gland on Pituitary Gonadotrophin Activity in the Rabbit, Endocrinology **34:** 90, 1944.
32. Mandl, A. M.: Factors Influencing Ovarian Sensitivity to Gonadotrophins, J. Endocrinol. **15:** 448, 1957.
33. Sommers, S. C., Chute, R. N., and Burt, A. S.: Cystic Hyperplasia of Endometrium and Breast in Mice With $I^{131}$ Induced Pituitary Adenomas (Abst.), Am. J. Path. **30:** 621, 1954.
34. Jackson, W. P. U.: Post-Thyroidectomy Hypothyroidism, Hypoparathyroidism, Exophthalmos and Galactorrhea With Normal Menstruation: Metabolic Response to Probenecid, J. Clin. Endocrinol. **16:** 1245, 1956.
35. Zondek, B., Bromberg, Y. M., and Rozin, S.: An Anterior Pituitary Hyperhormonotrophic Syndrome, J. Obst. & Gynaec. Brit. Emp. **58:** 525, 1951.
36. Burt, A. S., Reiner, L., Cohen, R. B., and Sniffen, R. C.: Klinefelter's Syndrome: Report of Autopsy With Particular Reference to Histology and Histochemistry of the Endocrine Glands, J. Clin. Endocrinol. **14:** 719, 1954.
37. Aron, M., and Benoit, J.: Influence de la castration sur le taux d'hormone préhypophysaire excito-sécrétrice de la thyroïde present dans le milieu intérieur, chez le cobaye, Compt. rend. Soc. de Biol. **83:** 784, 1931.
38. Fromm, G. A., Bur, E. B., Del Conte, E., Lascano, E. F., and Hecker, E.: Prepubertal Gonadal Insufficiency: Its Correlation With the Adrenals and Blood Thyrotropin, Acta endocrinol. **19:** 112, 1955.
39. Asper, S. P., and Wilson, E. H.: Coexisting Testicular Deficiency and Hyperthyroidism— By Design or By Chance, Tr. A. Am. Physicians **70:** 148, 1957.

40. von Basedow, C. A.: Die Glotzangen, Wchnschr. Gesammte Heilkunde **14:** No. 49, 769, 1848.
41. Rupp, J. J., and Paschkis, K. E.: Co-existence of Addison's Disease and Thyrotoxicosis, J. Clin. Endocrinol. **17:** 143, 1957.
42. Williams, R. H.: Thyroid and Adrenal Interrelations With Special Reference to Hypotrichosis Axillaris in Thyrotoxicosis, J. Clin. Endocrinol. **7:** 52, 1947.
43. Marine, D.: Remarks on Pathogenesis of Graves' Disease, Am. J. M. Sc. **180:** 767, 1930.
44. Roth, O.: Auftreten von Milchsekretion bei einem an akromegalie leidenden Patienten, Klin. Wchnschr. **55:** 305, 1918.
45. Rawson, R.: Personal communication.
46. McCullagh, E. P., Beck, J. C., and Schaffenburg, C. A.: Control of Diabetes and Other Features of Acromegaly Following Treatment With Estrogens, Diabetes **4:** 13, 1955.
47. Kovačić, N.: An Attempt to Inhibit Synthesis of Corticosteroid Hormones by Means of Diethylstilboestrol in a Case of Cushing's Syndrome. Preliminary Report, Acta med. scandinav. **164:** (Fasc. 6) 455, 1959.
48. Brown-Grant, K.: The "Feed-Back" Hypothesis of the Control of Thyroid Function, in Wolstenholme, G. E. W., and Millar, E. C. P., editors: The Ciba Foundation Colloquia on Endocrinology, vol. 10, Regulation and Mode of Action of Thyroid Hormones, Boston, 1957, Little, Brown & Company, p. 97.
49. Li, C. H.: Anterior Pituitary Gland Hormones (Part II), in Anson, M. L., Bailey, K., and Edsall, J. C. T., editors: Advances in Protein Chemistry, New York, 1955, Academic Press, Inc., p. 4.
50. Van Dyke, H. B., Adamsons, K., Jr., and Engel, S. L.: Aspects of the Biochemistry and Physiology of the Neurohypophyseal Hormones, in Pincus, Gregory: Recent Progress in Hormone Research, vol. 11, New York City, 1955, Academic Press, Inc., p. 4.

# *Pseudo-pseudohypoparathyroidism*

## *A disputable syndrome*

**C. Papadatos, M.D.,* and J. G. Alivisatos, M.D., M.Sc.**

ATHENS, GREECE

IN 1952 Albright, Forbes, and Henneman[1] described a 29-year-old single woman of short stature, with a "round" face, subcutaneous calcinosis, abnormal variation in the length of the fingers, and all the characteristics of pseudo-hypoparathyroidism, except that she had no laboratory evidence suggesting a parathyroid dysfunction. They applied the term of pseudo-pseudohypoparathyroidism "or a-hypocalcemic pseudohypoparathyroidism or more correctly a-hyperphosphatemic—pseudohypoparathyroidism."[1]

Further examples of pseudo-pseudohypoparathyroidism were soon recognized[2-8] and 9 additional cases have now been reported, all showing multiple defects, with brachydactylia caused by underdeveloped metacarpal bones the most outstanding and common abnormality.

It is the purpose of this paper to describe 2 additional cases of pseudo-pseudohypoparathyroidism studied at the Endocrine Department of Athens Polyclinic, and to call for a more critical definition of a condition with normal serum calcium and phosphorus concentrations and multiple congenital defects.

*From St. Sophies Children's Hospital of the University of Athens and the Endocrine Department of Athens Polyclinic.*

**Address, St. Sophies Children's Hospital, University of Athens, Athens, Greece.*

### CASE REPORTS

**Case 1.** X. G. (No. 12780), a 15-year-old white girl, was first examined in June, 1957, because of a congenital malformation of the toes and edema of the lower extremities. The patient was born in 1944. Pregnancy and delivery were uneventful and birth weight was 3.5 kilograms. Developmental history revealed that the patient was able to sit unsupported at the age of 1 year, walked at the age of 3 years, and could say a few words when she was 1 year old. Dentition was said to be normal. Past illnesses included measles, pertussis, and an extensive rash at the age of 6 months involving the entire body, which was not diagnosed. There was no family history of diabetes, endocrine disorders, or consanguinity. A sibling 2 years younger is somewhat taller than the patient. The parents have no abnormalities and there is no family history of seizures.

The patient's edema is not associated with any additional symptoms and at times, especially during menstruation, it becomes more pronounced. Menarche occurred in February of 1958 and menses have been

somewhat irregular as to duration and amount of bleeding.

*Physical examination:* Normal temperature, pulse, and respiration were observed. The blood pressure was 105/60. The weight in June of 1957 (age 13 years) was 105 pounds, and the height, 144 cm. In June of 1958 the weight was 130 pounds and the height 150 cm. The patient was well proportioned, with a normal amount of pubic hair and normal breast development. The face was broad and round (Fig. 1) and the neck short, she was pleasant and cooperative but obviously retarded mentally. The eyes were normal. Visual acuity was reduced and the funduscopic examination was negative. Slit lamp examination revealed a few lenticular and corneal opacities bilaterally. Dentition was normal. The lamina dura was intact. Heart, chest, and abdomen revealed no abnormalities. The genitals appeared normal, and neurological examination was essentially negative; neither the Chvostek nor the Trousseau sign could be elicited.

Fig. 1. Case 1. Note round face and absence of knuckles.

Fig. 2. Case 1. Feet of Patient X. G.

The appearance of the fingers and hands showed a brachydactylia of the third, fourth, and fifth metacarpals. When the fist was clenched it was evident that the knuckles of these fingers were absent. The fingernails were broad and short and the width/length ratio of the thumbnail was 3.5 on the right, and 2.4 on the left hand. Ray and Gardner[7] found that a group of normal children showed thumbnail width/length ratios which ranged between 1.1 and 1.4. The latter authors' case of "pseudo-pseudohypoparathyroidism" showed a thumbnail width/length ratio of 2.8. Our patient's feet showed mottling, with a nonpitting edema and a pronounced shortening of the second, third, fourth, and fifth toes (Fig. 2).

*Laboratory data.* The hemoglobin was 12.5 Gm., with a normal white count and normal differential. The urine contained no

albumin or sugar and only a rare white blood cell per high-power field. The nonprotein nitrogen was 25 mg. per 100 ml. A Wassermann test and tuberculin reaction were negative. Total serum protein was 6.9 Gm. per 100 ml. On a regular diet the serum calcium was on two occasions 10.3 and 10.5 mg. per 100 ml. and the phosphorus 5.3 and 5.9 mg. per 100 ml., respectively. Sodium was 143 and potassium 5.4 mEq. per liter.

An electrocardiogram was within normal limits. An x-ray of the skull showed no calcifications of basal ganglia; x-rays of the hand showed unusually short third, fourth, and fifth metacarpals (Fig. 3), and x-rays of the feet showed short second, third, and fourth metatarsals (Fig. 4). An electroencephalogram was interpreted as a borderline normal tracing with some background dysrhythmia. A psychometric examination disclosed an I.Q. of 58. An Ellsworth-Howard test was not done.

**Case 2.** O. P. (No. 31806), a 13-year-old girl, was first examined in June of 1958 because of obesity. The patient was born in December of 1946. Pregnancy and delivery were uneventful, and the birth weight was 8 pounds. Information was not available concerning her developmental history, but it was mentioned that the patient "was slow in walking and talking." Dentition was said to be normal.

Past illnesses included measles, mumps, and diphtheria. There was no family history

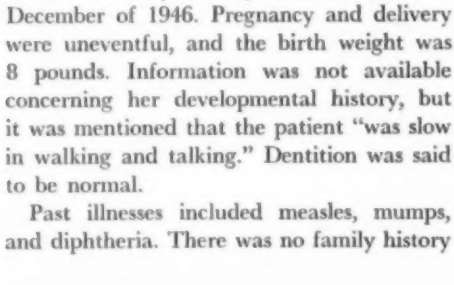


**Fig. 3.** Case 1. Note short third, fourth, and fifth metacarpal bones.

**Fig. 4.** Case 1. Short second, third, and fourth metatarsal bones.

of seizures, diabetes, or consanguinity. Of interest is the fact that the patient's grandmother has only three fingers on each hand with three metacarpals on the right and four on the left hand. The patient's mother has a normal hand on the left and three fingers with four metacarpals on the right, and the girl's brother has a syndactyly of the third, fourth, and fifth right fingers. The brother is younger and somewhat taller than the patient.

*Physical examination.* This was done in June of 1958 and revealed normal temperature, pulse, and respiration. The blood pressure was 115/80. The patient's weight in June of 1958 was 53.2 kilograms and height 132 cm. (The height age equaled 9½ years.) The patient was well developed with evident signs of pubescence. The breasts showed signs of development. She had a round facies with a short neck and multiple moles on the face and trunk (Fig. 5). The eyes were normal. Funduscopic examination disclosed no lenticular or corneal opacities. Dentition was normal. The lamina dura was intact. Examination of the heart, chest, and abdomen revealed no abnormalities. The genitals were normal. Neurological examination was negative, and Chvostek and Trousseau signs could not be elicited.

The appearance of the hands showed short and broad fingers, and when the fist was clenched no knuckles could be seen on

the third, fourth, and fifth metacarpals. The width/length ratio of the thumbnail was 2.3 on the right and 2.6 on the left hand. The fourth toe on each foot was short (Fig. 6).

*Laboratory data.* There was a hemoglobin of 11.8 Gm. with a normal white count and differential. Urinalysis was negative. Wassermann and tuberculin reactions were negative. Total serum proteins were 6.5 Gm. per 100 ml. On a regular diet the serum calcium was 9.5 and the phosphorus 4.0 mg. per 100 ml. An electrocardiogram was within normal limits. X-rays of the hands showed short third, fourth, and fifth metacarpal bones (Fig. 7), as well as a short fourth metatarsal bone (Fig. 8). A psychometric examination disclosed an I.Q. of 90. X-ray of the skull revealed no abnormal calcifications. An Ellsworth-Howard test was not done.

## DISCUSSION

Both patients described in this report fulfill most of the diagnostic criteria of pseudo-pseudohypoparathyroidism established in 1952 when Albright and associates[1] reported their original case. Metacarpal defects, obesity, "round" face, and normal serum calcium and phosphorus concentrations were noted. Calcinosis, which represents an inconstant finding in the cases reported in the literature, was absent in both of our patients.

The present patients as well as those whose cases were reported by Seringe and Tomkiewicz[6] have attained a fairly good height. Thus our patients belong in the twenty-fifth and third percentile group, respectively, for height, those described by Seringe and Tomkiewicz in the tenth and fiftieth group, respectively, and Ray and Gardner's[7] patient falls in the seventy-fifth percentile for height.

Of interest is the occurrence in our first patient of lenticular opacities. These are frequent in idiopathic hypoparathyroidism and pseudo-hypoparathyroidism and are considered as manifestations of hypocalcemia. Their presence in our case suggests that they may be due to a metabolic defect which is independent of parathyroid function.

**Fig. 5.** Case 2. Note round face and absence of knuckles.

**Fig. 6.** Feet (Case 2). Note similarity of feet in Cases 1 and 2.

Because of the lack of a potent parathyroid extract, an Ellsworth-Howard test was not performed. The meaning, however, of

**Table I.** Summaries of cases in literature

| *Cases* | *Sex* | *Age (years)* | *Height (cm.)* | *Calcification** | *Mental retardation* | *Obesity* | *Roundness of face* |
|---|---|---|---|---|---|---|---|
| Albright et al.[1] | F | 29 | 157 | + | | + | + |
| Miles and Elrick[2] | F | 24 | 145 | - | + | + | + |
| Roche[3] | M | 40 | 154 | - | | - | ± |
| McNeely et al.[4] | M | 24 | 156 | + | + | - | - |
| Wallach et al.[5] | M | 24 | 166 | + | - | | - |
| Seringe and Tomkiewicz[6] | F | 16 | 147 | - | + | + | ± |
| | F | 11 | 138 | - | + | + | + |
| Ray and Gardner[7] | F | 7½ | 128.5 | + | + | + | + |
| Smulyan and Raisz[8] | F | 65 | 142 | + | | - | + |
| Present cases | F | 15 | 152 | - | + | + | + |
| | F | 13 | 132 | - | + | + | + |

*+ = present; - = absent.

this test in normal individuals has been seriously challenged and its limitations have already been described.[9]

Table I summarizes the findings of the cases reported in the literature (including our 2). Thus dwarfism was seen in 6 of the 11 patients, calcinosis in 5, mental retardation in 7, obesity in 7, unquestionable roundness of the face in 7, and metacarpal defects were present in all the cases with the exception of the one reported by Wallach. It is well known, however, that this latter finding is not limited to pseudo-pseudohypoparathyroidism.[11-13] The significance of the brachymetacarpal stigma is as yet not clear.

In the past two years we have seen in the Endocrine Department of Athens Polyclinic unquestionably short metacarpals in one patient with ovarian agenesis, and in a second adult female with no obvious endocrine abnormalities. Three additional patients with mental retardation had short fourth and fifth metacarpal bones. We are at present investigating with objective criteria the significance of brachymetacarpal stigma in different conditions in combination with abnormal height, mental retardation, and single or multiple congenital defects. Direct measurements of metacarpals are being made by a roentgenographic technique. Preliminary results indicate that short metacarpals may occur without the other findings of pseudo-pseudohypoparathyroidism.[13] It is very unfortunate, as Fromm[10] has already pointed out, that the term pseudo-pseudohypoparathyroidism is used to denote a set of congenital abnormalities which may bear no relation to the function of the parathyroid glands.

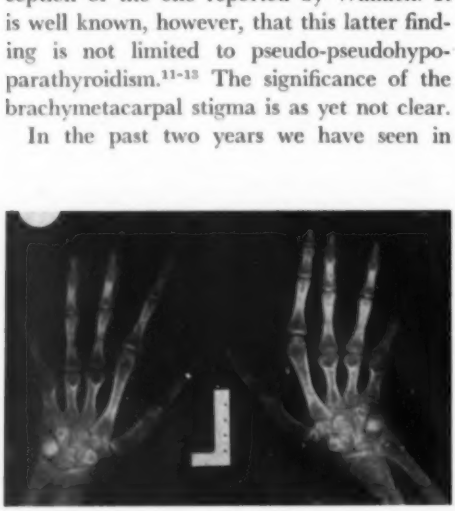


**Fig. 7.** Case 2. Short third, fourth, and fifth metacarpal bones.

van der Werff ten Bosch,[11] calling for an adequate definition of pseudo-pseudohypoparathyroidism, introduced the term "brachymetacarpal dwarfism," while McNeely and co-workers[4] preferred the designation of "dyschondroplasia with soft tissue calcifications and ossification and normal parathyroid function." The metacarpal and

| Round face | Short metacarpals | Short metatarsals | Lenticular opacities | Serum Ca (mg. %) | Serum P (mg. %) | Ellsworth-Howard test |
|---|---|---|---|---|---|---|
| + | + | + | - | 9.3 | 3.0 | Unresponsive |
| + | + | + | + | 11.0 | | Responsive |
| ± | + | - | + | 11.5 | 2.5 | Unresponsive |
| - | + | + | - | 8.6 | 3.7 | Responsive |
| - | - | - | - | 9.8 | 3.2 | ± |
| ± | + | | | 9.6 | 3.5 | Responsive |
| + | + | | | 9.7 | 3.6 | Responsive |
| + | + | + | | 9.5 | 7.4 | Unresponsive |
| + | + | | - | 10.0 | 3.5 | Unresponsive |
| + | + | + | + | 10.4 | 5.6 | |
| + | + | + | - | 9.5 | 4.0 | |

metatarsal defects may represent a genetic abnormality not necessarily related to parathyroid function.

Serum calcium and phosphorus values have been defined as normal in pseudo-pseudohypoparathyroidism. The patient described by Ray and Gardner,[7] however, presented an elevated concentration of inorganic phosphorus in the serum.*

Review of the Ellsworth-Howard tests reported in 2 papers revealed that in these 2 instances the response of phosphate excretion to injection of parathyroid extract was either slight or nil, while the same extract was potent in normal controls.[5, 8] What then constitutes the basic entity of pseudo-pseudohypoparathyroidism? It seems reasonable to suppose, as Smulyan and Raisz[8] point out, that some of the reported cases represent intermediate conditions between patients with pseudo-pseudohypoparathyroidism and pseudohypoparathyroidism. On the basis, therefore, of the diversity of clinical findings in the reported cases of pseudo-pseudohypoparathyroidism and pseudohypoparathyroidism, we feel that one is not justified in defining pseudo-pseudohypoparathyroidism as a syndrome distinct from pseudohypoparathyroidism.

*Special Editor's note: The patient of Ray and Gardner[7] showed an elevated serum inorganic phosphorus and normal calcium in December, 1956. When retested in June 1959, the serum inorganic phosphorus was still elevated but the serum calcium had fallen to a value of 6.7 mg. per 100 ml. She had, apparently, shifted from pseudo-pseudo- to pseudo-hypoparathyroidism in this time interval. The limitations of this nomenclature are obvious.

## SUMMARY

Two cases of the so-called syndrome of pseudo-pseudohypoparathyroidism are described.

Metacarpal and metatarsal defects may be seen not only in pseudo-pseudohypoparathyroidism, but are occa-

Fig. 8. Case 2. Short fourth metatarsal bone.

sionally encountered in patients with multiple congenital defects.

In view of the diversity of clinical findings and the lack of laboratory evidence, we consider that one is not justified in defining pseudo-pseudohypoparathyroidism as an entity distinct from pseudohypoparathyroidism.

## REFERENCES


1. Albright, F., Forbes, O. P., and Henneman, P. H.: Pseudo-pseudohypoparathyroidism, Tr. A. Am. Physicians **65:** 337, 1952.
2. Miles, J., and Elrick, H.: Pseudo-pseudohypoparathyroidism: Report of a New Case, J. Clin. Endocrinol. **15:** 576, 1955.
3. Roche, M.: A Case of Pseudo-pseudohypoparathyroidism, J. Clin. Endocrinol. **15:** 964, 1955.
4. McNeely, W. F., Raisz, L. G., and LeMay, M.: Dyschondroplasia With Soft Tissue Calcification and Ossification and Normal Parathyroid Function (Pseudo-pseudohypoparathyroidism), Am. J. Med. **21:** 649, 1956.
5. Wallach, S., Englert, E., Jr., and Brown, H.: The Syndrome of Pseudo-pseudohypoparathyroidism, A. M. A. Arch. Int. Med. **98:** 517, 1956.
6. Seringe, P., and Tomkiewicz, S.: La dystrophie d'Albright (Pseudo-pseudohypoparathyroidism de cet auteur), Semaine Hôp. de Paris **33:** 1092, 1957.
7. Ray, E. W., and Gardner, L. I.: Pseudo-pseudohypoparathyroidism in a Child: Report of the Youngest Case, Pediatrics **23:** 520, 1959.
8. Smulyan, H., and Raisz, L. G.: Pseudo-pseudohypoparathyroidism With Unusual Features, J. Clin. Endocrinol. **19:** 478, 1959.
9. MacGregor, M. E., and Whitehead, T. P.: Pseudo-hypoparathyroidism: A description of Three Cases and a Critical Appraisal of Earlier Accounts of the Disease, Arch. Dis. Childhood. **16:** 398, 1956.
10. Fromm, G. A.: Concerning the Term "Pseudo-pseudohypoparathyroidism," J. Clin. Endocrinol. **16:** 293, 1956.
11. van der Werff ten Bosch, J. J.: The Syndrome of Brachymetacarpal Dwarfism (Pseudo-pseudohypoparathyroidism) With and Without Gonadal Dysgenesis, Lancet **1:** 69, 1959.
12. Lomas, J. J. P., and Boyle, A. C.: Osteochondrodystrophy (Morquio's Disease) in Three Generations, Lancet **2:** 430, 1959.
13. Alivisatos, J. G., Arzimanoglou, A., Papadatos, C., and Chiotaki, P.: The Brachymetacarpal Stigma. The Significance of This Sign and Its Relationship to Pseudo-pseudohypoparathyroidism. (To be published.)

# A congenital, familial syndrome of adrenocortical insufficiency without hypoaldosteronism

Robert S. Stempfel, Jr., M.D.,* and Frank L. Engel, M. D.

DURHAM, N. C.

FAMILIAL adrenocortical insufficiency occurring in infancy is usually associated with congenital adrenal hyperplasia, an inborn error in steroidogenesis resulting in progressive virilization.[1] Congenital adrenal hypoplasia is an extremely rare condition[2-6] which at times may coexist with hypoplasia of the pituitary gland,[7-9] the latter having been reported in twin siblings.[8] The familial syndrome of adrenal insufficiency, hypoparathyroidism, and superficial moniliasis is the subject of a recent review.[10] While familial Addison's disease in the absence of other endocrinopathy is also rare, sporadic reports of well-established cases have been appearing with increasing frequency.[11-15] Recently Shephard and colleagues[16] described the condition in infancy, while Mitchell and Rhaney[6] reported the first cases of adrenal hypoplasia in siblings in whom pituitary hypoplasia was not a finding.

With the development of more adequate methods for analysis of adrenal steroids, it has become obvious that the dualism in adrenocortical function may be reflected in states of adrenal deficiency in man. Specific aldosterone deficiency has been recorded in at least three instances,[17-19] although the criteria for accepting the diagnosis in each case have not been fully acceptable to all.[20] The cases reported by Shephard and colleagues[16] represent the first recorded examples of primary adrenal insufficiency in which production of hydrocortisone has been shown to be deficient, while secretion of aldosterone was grossly intact. This same situation occurs, secondarily, in adenohypophyseal deficiency and, transiently, following prolonged treatment with cortisone and its analogs.

The purpose of the present report is to describe another example of familial adrenal insufficiency in which the production of aldosterone is intact, but which differed from Shephard's case in that the disorder presumably is congenital, since it became clinically apparent during the neonatal period.

*From the Departments of Pediatrics and Medicine and the Endocrine Division, Duke University Medical Center, Durham, N. C.*

*This study was supported in part by grants (A-2819 and 2A-5074) from the Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.*

*Address, Division of Pediatrics, Duke University Medical Center, Durham, N. C.*

## CASE REPORT

This 25-month-old white boy was seen for the first time at the Duke University Medical Center for endocrine evaluation on Nov. 27, 1957 (Duke Hospital Unit No. E-67658).

**Family history.** The mother at 33 was in good health; the father was 32 and also well except for mild hay fever. One sibling, a male 6 years old, was in good health. Another male sibling, born of a full-term pregnancy 16 months prior to the birth of the patient, died at 18 hours. No adrenal tissue could be found on postmortem examination at the Oakland Children's Hospital, Oakland, California. The remainder of family history was noncontributory.

**Past history.** The patient was born at St. Luke's Hospital, Kansas City, Missouri. The pregnancy had been normal and full term. The birth weight was 8.25 pounds and no immediate neonatal abnormalities were noted. Shortly after discharge from the hospital, feeding difficulties developed which were associated with occasional vomiting and by 12 days of age there had been a loss of weight to 6.87 pounds. At that time the patient was admitted to Menorah Hospital, Kansas City,* with extreme dehydration and pallor and with the following serum electrolytes: sodium, 132 mEq. per liter; potassium, 7.6 mEq. per liter; and $CO_2$, 11.2 mEq. per liter. These findings, in an infant whose sibling had adrenal aplasia, strongly suggested adrenal insufficiency and the child was placed on cortisone, intravenous fluids, and antibiotics. Marked improvement followed. Skull, chest, and gastrointestinal x-rays are said to have been within normal limits. A trial on ACTH after withdrawal of cortisone resulted in prompt exacerbation of the original clinical picture, and blood chemistries at that time showed serum sodium, 116 mEq. per liter; potassium, 9.0 mEq. per liter; and $CO_2$, 16.5 mEq. per liter. The provisional diagnosis of congenital adrenal hyperplasia of the salt-losing type was then made and corticoid therapy reinstituted. The child apparently did well on varying amounts of intramuscular cortisone and at 2 months of age was discharged from the hospital weighing 9.34 pounds and receiving 50 mg. of oral cortisone acetate daily.

At 3 months of age he was again hospitalized during an episode of "flu" which was complicated by diarrhea, dehydration, listlessness, and ashen pallor, but his response to fluid and corticoid therapy was apparently adequate.

Between 3 and 4 months of age cortisone was reduced to 37.5 mg. by mouth daily. Photographs taken at that time show the typical facies of hyperadrenocorticism. The parents state that from 4 to 8 months of age the child had "very poor muscle tone," that he rarely cried, and that there was frequent lethargy. At 8 months of age the dosage of cortisone was further reduced to 25 mg. daily in divided doses, but little change in facial appearance was noticeable by 14 months of age. At that time the hormone was gradually adjusted to a dosage of 12.5 mg. daily over the next 3 months. In addition to the cortisone, the patient also received intramuscular desoxycorticosterone, 0.5 mg. every 7 to 10 days, with dietary salt supplementation during the first 18 months of life. Throughout this period the child did reasonably well, except for several episodes of diarrhea and vomiting which the parents relate to the intake of certain foods. He is also said to have become lethargic and irritable during the hot summer weather. There was a definite delay in statural growth (Fig. 1), and for a considerable period after cortisone was reduced to 12.5 mg. daily, he still presented the appearance one associates with Cushing's syndrome.

Motor development was also moderately slow: He rolled to a prone position at 7 months, sat alone at 9 months, pulled himself up at 12 months, walked at 16 months, and spoke a few words at 24 months. Teething is said to have begun at 3 months.

At 22 months of age the child was admitted to the Pediatric Service of the University of California Medical Center where he was seen in consultation by Dr. Vincent

*Information regarding this hospitalization was provided by Dr. M. D. Bordy, Kansas City, Mo.

**Table I**

| *Cortisone acetate (mg./day)* | *17-ketosteroids (mg./24 hours)* | *17-hydroxycorticosteroids (mg./24 hours)* |
|---|---|---|
| 30 | 0.6 | 11.4 |
| 30 | 1.1 | 8.4 |
| 10 | 0.6 | 4.4 |
| . . . . ACTH-gel, intramuscularly, 40 U.S.P. units twice daily for 5 days: | | |
| 10 (third day ACTH) | 0.5 | 2.5 |
| 10 (fifth day ACTH) | 1.1 | 10.8 |

**Table II**

| *Age (months)* | *Treatment* | *17-ketosteroids* (mg./24 hours)* | *17-hydroxycorticosteroids† (mg./24 hours)* |
|---|---|---|---|
| 27 | Cortisone, 5 mg. daily | 1.3 | 2.2 |
| 28 | Cortisone, 5 mg. daily | 1.1 | 1.5 |
| 30 | Cortisone, 5 mg. daily<br>ACTH-gel intramuscularly 20 U./day, third day | 1.3 | 1.6 |
| | Cortisone, 5 mg. daily<br>ACTH-gel intramuscularly 20 U./day, sixth day | 1.8 | 2.0 |
| 32 | Cortisone, 5 mg. daily<br>Zn-ACTH intramuscularly 40 U./day, fourth day | 1.5 | 1.1 |
| | Cortisone, 5 mg. daily<br>Zn-ACTH intramuscularly 40 U./day, ninth day | 1.3 | 1.0 |
| | Cortisone, 5 mg. daily<br>Zn-ACTH intramuscularly 40 U./day, twelfth day | 1.8 | 1.3 |
| | | *Circulating eosinophils/cu. mm.* | *Plasma‡ 17-hydroxycorticosteroids* |
| 33 | Before administration of ACTH;<br>14 hours after last dose cortisone | 650 | 0 |
| | 2 hours after 40 U. ACTH-gel intramuscularly | 817 | 0 |

*Method of Holtorff and Koch.[24]

†Method of Reddy, Jenkins, and Thorn.[23]

‡Method of Silber and Porter.[27]

C. Di Raimondo. His findings were as follows:

The patient was alert and active; his appearance was entirely normal except that at a chronologic age of 22 months, his height was that of 11 months. No abnormal pigmentation was noted and the child was not "Cushingoid" in appearance. Physical findings were otherwise within normal limits. Laboratory investigation revealed a bone age of 6 to 12 months. The skull x-ray was negative. The hemoglobin was 14.5 Gm. per cent and the white blood cell count per cubic millimeter was 13,450 with 5 per cent eosinophilia. Urinalysis was negative. Serum electrolytes and calcium were within the normal range. Protein-bound iodine was 5.5 mcg. per cent and serum cholesterol 233 mg. per cent. $I^{131}$ uptake was in midnormal range (23 per cent at 24 hours). The results of studies on urinary steroid excretion are given in Table I.

The patient was discharged on his sixteenth hospital day after an uneventful course and was put on a regular diet without added salt, without DOCA, and with cortisone acetate, 5 mg. twice daily given orally. He continued to do quite well on this regimen and underwent a further reduction in cortisone dosage to 7.5 mg. daily about 1 month prior to his first visit to Duke Hospital.

**Physical examination.** On examination at 25 months he was alert, active, and pleasant, with a slight to moderate speech retardation. He had normal proportions but his height of

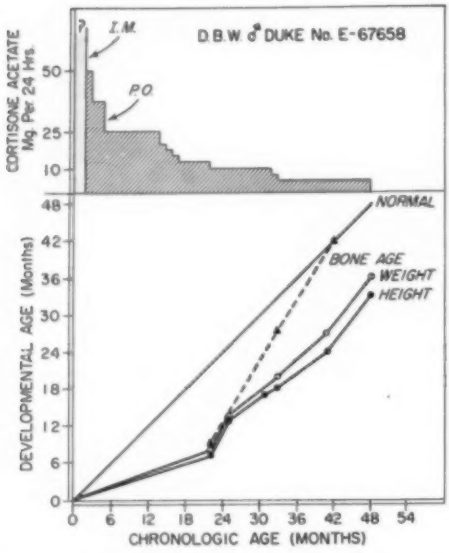



**Fig. 1.** Pattern of weight, height, and osseous development versus cortisone administration.

30 inches was that of 14 months. He weighed 22 pounds. Pulse was 70, respiration 18, and blood pressure 80/60 mm. Hg. The skin was warm and smooth without abnormal pigmentation. No local or generalized lymphadenopathy was present but tonsils showed moderate hypertrophy. There were 8 upper and 8 lower deciduous teeth. Head, eyes, ears, nose, and throat were otherwise not remarkable. The chest was clear to percussion and auscultation, and there were no gross cardiac abnormalities. The abdomen was soft and without palpable masses. No anomalies of the genitalia were noted, and while the child was sexually infantile, the testes were or normal size, shape, and consistency. No edema was present and the peripheral pulses were easily palpable. Neurological examination was entirely negative except for the delayed speech.

**Laboratory studies.** Routine laboratory studies on this visit were within normal limits. Bone age was approximately 2¼ years at the chronologic age of 33 months. The dosage of cortisone acetate was reduced to 2.5 mg. grams twice daily given orally, and arrangements were made to study adrenal function on an outpatient basis. The data obtained during the next 8 months are listed in Table II and tend to indicate a complete lack of responsiveness to intramuscularly administered ACTH. It was noted that at 22 months of age the patient showed an apparent rise in corticoid excretion after 5 days of administration of ACTH. However, there had been no increase after 3 days of ACTH and vigorous attempts to elicit the response 8 months later were completely unsuccessful. It is suggested that this isolated value was in error.

Because of the above findings, at the age of 41 months the patient was admitted to Duke Hospital for the first time for a more complete evaluation, cortisone having been discontinued 24 hours prior to admission. Physical examination was essentially as before, revealing an alert, healthy appearing child who was small for the stated age and who had moderately retarded speech, but who had no other gross abnormalities. Temperature, pulse and respiration were normal and blood pressure was 90/60 mm. Hg. Body measurements obtained on admission are recorded in Table III and a photograph made at that time is shown in Fig. 2.

Laboratory study revealed a hemoglobin of 12.7 Gm. per cent and a white blood cell count of 9,400 cubic millimeter with 10 per cent eosinophilia. Stools and urinalysis were negative (urine, specific gravity of 1.012). Fasting blood sugar was 93 mg. per cent, serum calcium 9.4 mg. per cent, and serum phosphorus 4.6 mg. per cent.

X-rays showed a bone age of 3.5 years (all centers surveyed). Films of the chest and abdomen showed no gross calcification of the adrenal glands and were otherwise not remarkable.

On admission the patient was placed on a diet of 800 calories which contained 4 mEq. of sodium, but it is probable that he received slightly less than this amount in view of his poor appetite during his first few hospital days. After 5 days there was no loss of weight and no significant fall in blood pressure. Serum electrolytes and urinary

sodium, potassium, and chloride excretion are listed in Table IV. The data indicate that the child was able to maintain a positive sodium balance throughout the period of observation, but it must not be overlooked that sodium restriction was of only a very modest degree and that a more vigorous deprivation might not have been tolerated as well. Ideally, the study would be carried out with a sodium intake of approximately one-fourth that actually employed. The relatively low control serum sodium level is unexplained, but the value had undergone a slight increase after 4 days on the low-sodium diet, and in both instances the serum potassium and chloride were normal. Urinary aldosterone determinations were performed through the courtesy of Dr. Jerome W. Conn, University of Michigan. The baseline level of 5.0 Mcg. per 24 hours is considered to be within the normal range in that laboratory, but the value of 6.0 Mcg. per 24 hours, after sodium restriction, is thought to represent a hyporesponse. Again, this may well indicate inadequate salt deprivation or an inability to respond to the stimulus. In any event, however, the conclusion may be drawn that production of aldosterone was intact and that the child did not have frank hypoaldosteronism.

On the second hospital day, 48 hours after the withdrawal of cortisone and after overnight deprivation of water, the patient was given an oral water load of 20 ml. per kilogram of body weight (260 ml.) within a period of 30 minutes. During the next 4 hours he excreted a total of 147 ml., representing 57 per cent of the administered load, which was well below the expected normal of greater than 70 per cent. Because of the anticipated studies on hormonal excretion, this test was not repeated after the administration of cortisone.

**Table III.** Body measurements at a chronologic age of 41 months

| | *Patient* | *Average for height** | *Average for age** |
|---|---|---|---|
| Weight (pounds) | 28.6 | 27.2 | 34.0 |
| Height (inches) | 34.0 | Average height for 24 months | 38.6 |
| Span | 34.2 | 32.7 | 37.5 |
| Upper segment | 19.6 | 20.1 | 22.1 |
| Lower segment | 14.4 | 13.9 | 16.5 |
| U/L ratio | 1.35 | 1.45 | 1.31 |
| Head circumference | 19.7 | 19.4 | 20.0 |
| Chest circumference | 20.3 | 19.7 | 21.0 |
| Abdomen | 20.3 | 18.9 | 19.8 |

*Average values after Wilkins.[1]

**Fig. 2.** D. W. (E-67658) at 3½ years of age while receiving oral cortisone acetate, 2.5 mg. twice daily. Bone age was 3½ years. His height was that of a 2 year old.

Studies of urinary steroid excretion as well as plasma 17-hydroxycorticosteroid determinations, were performed again before and after ACTH administration during the period of sodium restriction. These data are included in Table IV. It will be noted that there was no discernible response in the urinary excretion or plasma concentration of free or conjugated 17-hydrocorticosteroids to the intramuscular or intravenous administration of ACTH. Excretion of urinary 17-ketosteroid and urinary pregnanetriol likewise underwent no measurable change in response to this stimulation.

Determination of 17-hydroxycorticosteroids by the method of Reddy, Jenkins, and

**Table IV**

| *Hosp. day* | *Remarks* | *Urinary steroid excretion** | | | | |
|---|---|---|---|---|---|---|
| | | *mg. per 24 hours* | | | | |
| | | *17-ketosteroids* | *17-hydroxycorticosteroids* | | *Pregnanetriol* | *Aldosterone‡ (mcg./24 hours)* |
| | | | *R-J-T* | *G-N* | | |
| 1 | 800-calorie diet, 43 mEq. of sodium§ | | | | | 5.0 |
| 2 | | 1.2 | 0.2 | 0 | 0 | |
| 3 | 40 U. ACTH-gel, intramuscularly | 1.8 | 0.3 | 0 | 0 | |
| 4 | 40 U. ACTH-gel, intramuscularly | 1.3 | 0.7 | 0 | 0 | |
| 5 | 40 U. ACTH-gel, intramuscularly | 1.2 | 0.4 | 0 | 0 | |
| 6 | 15 U. ACTH intravenously (6 hours) | 1.2 | 0.4 | 0 | 0 | 6.0 |
| | Control | | | | | 0 |
| | 2 hours | | | | | 0 |
| | 4 hours | | | | | 0 |
| | 6 hours | | | | | 0 |
| 7 | Regular diet, cortisone 2.5 mg. every 12 hours, orally | 0.9 | 0.4 | 0 | | |

*Urinary steroids determined by the following methods: 17-hydroxycorticosteroids (*R-J-T*) – Reddy, Jenkins, and Thorn.[21]
17-hydroxycorticosteroids (*G-N*) – Glenn and Nelson.[22]
17-Ketosteroids – Holtorff and Koch.[24]
Pregnanetriol – Bongiovanni and Eberlein.[26]

Thorn[21] indicated the possible presence of measurable Porter-Silber chromogens, but multiple determinations by the method of Glenn and Nelson[22] failed to confirm this observation. Accordingly, washed extracts of larger volumes of post-ACTH, B-glucuronidase-hydrolyzed urine (⅓ total 24-hour volume) were processed according to the technique of Nelson and Samuels[23] for the estimation of plasma corticoids. The results obtained suggest that the total corticoid excretion was less than 0.1 Mcg. per 24 hours after administration of ACTH.

The patient was discharged on the seventh hospital day at the conclusion of the above studies and was instructed to continue cortisone acetate, orally, in a dosage of 2.5 mg. twice daily. He was last seen at the age of 48 months having been well in the interim except for one bout of tonsillitis from which he recovered uneventfully. At that time he was 36 inches tall and had gained 4 pounds (Fig. 1).

## DISCUSSION

The diagnosis of some form of adrenocortical insufficiency was suspected shortly after birth in this case, largely because of the history of sudden death in the sibling and a postmortem examination in that child which apparently failed to detect adrenal tissue. It is not unreasonable to assume that both siblings were affected by variants of the same disease process.

The patient responded well to treatment with cortisone and desoxycorticosterone in the neonatal period but underwent prompt regression when ACTH was administered after corticoid withdrawal. It is apparent from the history, photographs, and the documented pattern of statural growth and osseous maturation that, at least during the first 18 months of life, cortisone was administered in amounts far in excess of the child's replacement needs. Upon the reduction of the dosage of cortisone to within the physiologic range, there was a striking augmentation of the rate of osseous maturation, but statural growth continues to show a rather marked deficit on subphysiologic corticoid therapy, in contrast to the slightly advanced rate of growth sometimes seen in adrenal insufficiency.[13, 16] It is probable, however, that not enough time has elapsed to allow an obvious compensatory statural growth spurt to occur. The possibility that this pattern of linear growth represents another congenital defect cannot be unequivocally ruled out, although no abnormalities of weight or size were noted at birth.

| *Plasma†* *Mcg. % 17-hydroxycorticosteroids* | | *Electrolytes* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Serum (mEq./L.)* | | | *mg. %* | *Urine (mEq./24 hours)* | | |
| *Free* | *Conjugates* | *Sodium* | *Potassium* | *Chloride* | *Blood urea nitrogen* | *Sodium* | *Potassium* | *Chloride* |
| | | 128 | 4.2 | 97.1 | 20 | 27.6 | 25.9 | 18.2 |
| | | | | | | 28.8 | 33.7 | 24.9 |
| | | | | | | 20.5 | 30.8 | 20.1 |
| | | 133 | 4.4 | 99.8 | 20 | 14.3 | 33.5 | 19.6 |
| | | | | | | 20.5 | 13.7 | 13.2 |
| 0 | 0 | | | | | | | |
| 0 | 0 | | | | | | | |
| 0 | 0 | | | | | | | |
| 0 | 0 | | | | | | | |

†Plasma free 17-hydroxycorticosteroids determined by the method of Nelson and Samuels[23] and plasma 17-hydroxycorticosteroids conjugates by the method of Bongiovanni and Eberlein.[25]

‡Urinary aldosterone determinations were performed in the laboratory of Dr. Jerome W. Conn, University of Michigan, Ann Arbor, Mich.

§Cortisone acetate (oral) discontinued 24 hours prior to admission.

While, in retrospect, the diagnosis of congenital adrenal hyperplasia was made on somewhat inadequate grounds during the neonatal period, this possibility was no longer entertained when withdrawal of cortisone and prolonged administration of ACTH failed to produce a measurable rise in the urinary excretion of 17-ketosteroids or of pregnanetriol.

The familial syndrome of Addison's disease and hypoparathyroidism with or without superficial moniliasis is a consideration, but in patients so affected, the moniliasis and/or hypoparathyroidism characteristically antedates the development of adrenal insufficiency,[10] findings which were not observed in the present case. Serum calcium determinations have thus far been consistently within the normal range and there has been no clinical evidence of moniliasis.

Congenital pituitary hypoplasia with adrenal hypoplasia of the type described by Mosier[8] cannot be definitively excluded on the basis of the studies included here. Autopsy findings in regard to the pituitary gland in the sibling are lacking, but the developmental picture, the complete absence of adrenal response to ACTH, and the studies of normal thyroid function are not consistent with the findings in clinically assessable forms of hypopituitarism known at this time. However, the familial absence of an adrenotropic factor produced by the pituitary and necessary to the normal development of the fetal adrenal cortex is worthy of consideration. It is also noteworthy that patients with deficient corticoid production secondary to hypopituitarism respond to sodium restriction with an increase in the excretion of aldosterone.[28]

With neonatal adrenal insufficiency and a history suggestive of adrenal aplasia in a sibling, it is probable that this child has congenital, familial adrenal hypoplasia involving primarily those glandular elements responsible for the production of glucocorticoids. Hyperpigmentation, usually but not always seen in neonatal adrenal insufficiency, was not present here. It might be questioned whether the absence of hyperpigmentation in such cases is an indirect indication of dysfunction of the pituitary. The case of congenital adrenal hypoplasia described by Mitchell and Rhaney,[6] however, showed no pigmentary changes and was found to have an histologically normal pituitary.

The contention that the patient described represents an example of an isolated defect in the production of 17-hydroxycorticosteroids is supported by data which reveal an

apparently complete inability to produce these substances in response to intravenous and prolonged intramuscular administration of ACTH, a similar lack of response in the excretion of 17-ketosteroids or fall in circulating eosinophils, and the failure to excrete adequately a water load, in spite of baseline levels of aldosterone excretion which fell within the normal range. The ability to tolerate a period of moderate salt restriction while maintaining a positive sodium balance was also observed, while there was little if any change in aldosterone excretion at the conclusion of this test. However, it is possible that the regimen employed served as an inadequate stimulus for aldosterone production. An attractive postulation is that the mechanism by which the hormone is produced was under constant maximal stimulation and that the gland actually lacked the capacity for additional response. It might be further postulated that production of aldosterone was primarily limited because of deficient precursor for its formation.

It is of great interest that the presence of a salt-losing condition was clearly established during the neonatal period, especially since the child had no such episodes later when treated with cortisone alone. Blizzard and co-workers[29] have shown that aldosterone responsiveness may be limited to some extent in the normal newborn infant, although the data are as yet inconclusive. Since it is possible that production of aldosterone was subnormal in this patient, and perhaps to a much greater degree during early infancy, one might wonder to what extent the salt-losing symptoms were influenced by a profound glucocorticoid deficiency.

While little is known of the effects of massive corticoid administration upon future adrenal function, especially when this therapy is imposed upon the neonate, virtually nothing is known of the effect of prolonged ACTH suppression upon an already defective adrenal cortex. It is tempting to speculate in regard to possible implications in the present case, but the history of an adrenal anomaly in the sibling would tend to minimize the importance of such considerations.

It is extremely unlikely that variants of adrenal dysgenesis would occur in siblings on the basis of pure chance. Whether the picture is due to primary fetal adenohypophyseal dysfunction, an antiadrenal autoimmune mechanism, or to a toxic or infectious agent is entirely conjectural, but that it could be associated with an inborn error in steroidogenesis (e.g., a block in 17-hydroxylation) awaits further study in the patient described here.

## SUMMARY

Congenital adrenocortical insufficiency is reported in a boy whose male sibling died shortly after birth with no identifiable adrenal tissue at postmortem examination. Evidence is presented to support the belief that this represents a syndrome of congenital, familial, selective hydrocortisone deficiency. Clinical and laboratory investigation of the surviving sibling indicated an apparently complete absence of 17-hydroxycorticosteroid production, even after prolonged ACTH administration, in spite of normal aldosterone excretion while on *ad lib.* sodium intake.

Suggestions as to a possible etiology in this condition are discussed.

## ADDENDUM

Paper chromatographic fractionation of pooled urinary extracts (both before and after administration of ACTH) failed to reveal the presence of tetrahydrometabolites of compounds B or A and the absence of 17-hydroxycorticosteroids was reconfirmed. These findings tend to exclude defective 17-hydroxylation as the principal factor in this patient's adrenal insufficiency.

## REFERENCES

1. Wilkins, L.: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence, ed. 2, Springfield, Ill., Charles C. Thomas, Publisher, 1957.
2. Sikl, H.: Addison's Disease Due to Congenital Hypoplasia of the Adrenals in an Infant Aged 33 Days, J. Path. & Bact. **60:** 323, 1948.
3. Deamer, W. C., and Silver, H. K.: Abnormalities in the Secretion of the Adrenal Cortex During Early Life, J. PEDIAT. **37:** 490, 1950.

4. Williams, A., and Robinson, M. J.: Addison's Disease in Infancy, Arch. Dis. Childhood **31:** 265, 1956.
5. Harlem, O. K., and Myhre, E.: Congenital Adrenal Hypoplasia: Report of a Case With the Characteristic Clinical Features of Dysadrenocorticism and Autopsy Findings of Extreme Hypoplasia of the Adrenal Glands, A. M. A. J. Dis. Child. **94:** 696, 1957.
6. Mitchell, R. G., and Rhaney, K.: Congenital Adrenal Hypoplasia in Siblings, Lancet **1:** 488, 1959.
7. Blizzard, R. M., and Alberts, M.: Hypopituitarism, Hypoadrenalism, and Hypogonadism in the Newborn Infant, J. Pediat. **48:** 782, 1956.
8. Mosier, H. D.: Hypoplasia of the Pituitary and Adrenal Cortex: Report of Occurrence in Twin Siblings and Autopsy Findings, J. Pediat. **48:** 633, 1956.
9. Brewer, D. B.: Congenital Absence of the Pituitary Gland and Its Consequences, J. Path. & Bact. **73:** 59, 1957.
10. Whitaker, J. A., Landing, B. H., Esselborn, V. M., and Williams, R. R.: The Syndrome of Familial Juvenile Hypoadrenocorticism, Hypoparathyroidism and Superficial Moniliasis, J. Clin. Endocrinol. **16:** 1374, 1956.
11. Moehlig, R. C.: Addison's Disease Followed for Nine Years: Case Report With Autopsy, J. Clin. Endocrinol. **7:** 134, 1947.
12. Berlin, R.: Addison's Disease: Familial Incidence and Occurrence in Association With Pernicious Anemia, Acta med. scandinav. **144:** 1, 1952.
13. Bie, J.: Morbus Addisonii hos børn: forekomst hos to søskende, Nord. med. **55:** 193, 1956.
14. DeCock, J., DeGraeff, J., Smeenk, D., and Querido, A.: Chronic Primary Adrenal Insufficiency, Nederl. tijdschr. geneesk. **101:** 2426, 1957.
15. Meakin, J. W., Nelson, D. H., and Thorn, G. W.: Addison's Disease in Two Brothers, J. Clin. Endocrinol. **19:** 726, 1959.
16. Shephard, T. H., Landing, B. H., and Mason, D. G.: Familial Addison's Disease: Case Reports of Two Sisters With Corticoid Deficiency Unassociated With Hypoaldosteronism, A. M. A. J. Dis. Child. **97:** 154, 1959.
17. Hudson, J. B., Chobanian, A. V., and Relman, A. S.: Hypoaldosteronism: A Clinical Study of a Patient With an Isolated Adrenal Mineralocorticoid Deficiency, Resulting in Hyperkaliemia and Stokes-Adams Attacks, New England J. Med. **257:** 529, 1957.
18. Pedersen, J., and Sondergaard, E.: Partial Addison's Disease, Acta endocrinol. **24:** 370, 1957.
19. Skanse, B., and Hökfelt, B.: Hypoaldosteronism With Otherwise Intact Adrenocortical Function, Resulting in a Characteristic Clinical Entity, Acta endocrinol. **28:** 29, 1958.
20. Hills, A. G.: Selective Hypoaldosteronism (editorial), Am. J. Med. **26:** 503, 1959.
21. Reddy, W. J.: Modification of Reddy-Jenkins-Thorn Method for Estimation of 17-hydroxycorticosteroids in Urine, Metabolism **3:** 489, 1954.
22. Glenn, E. M., and Nelson, D. M.: Chemical Method for the Determination of 17-hydroxycorticosteroids and 17-ketosteroids in Urine Following Hydrolysis With B-glucuronidase, J. Clin. Endocrinol. **13:** 911, 1953.
23. Nelson, D. H., and Samuels, L. T.: A Method for the Determination of 17-hydroxycorticosteroids in Blood: 17-hydroxycorticosterone in the Peripheral Circulation, J. Clin. Endocrinol. **12:** 519, 1952.
24. Holtorff, A. F., and Koch, F. C.: The Colorimetric Estimation of 17-ketosteroids and Their Application to Urine Extracts, J. Biol. Chem. **135:** 377, 1940.
25. Bongiovanni, A. M., and Eberlein, W. R.: Determination, Recovery, Identification and Renal Clearance of Conjugated Adrenal Corticoids in Human Peripheral Blood, Proc. Soc. Exper. Biol. & Med. **89:** 281, 1955.
26. Bongiovanni, A. M., and Eberlein, W. R.: Critical Analysis of Methods for Measurement of Pregnane-3-alpha, 17-alpha, 20-alpha-triol in Human Urine, Analyt. Chem. **30:** 388, 1958.
27. Silber, R. H., and Porter, C. C.: The Determination of 17,21-dihydroxy-20-ketosteroids in Urine and Plasma, J. Biol. Chem. **210:** 923, 1954.
28. Liddle, G. W., Duncan, L. E., Jr., and Bartter, F. C.: Dual Mechanism Regulating Adrenocortical Function in Man, Am. J. Med. **21:** 380, 1956.
29. Blizzard, R. M., Liddle, G. W., Migeon, C. J., and Wilkins, L.: Aldosterone Excretion in Virilizing Adrenal Hyperplasia, J. Clin. Invest. **38:** 1442, 1959.

# *Evidence for and against the existence of a salt-losing hormone*

R. Klein, M.D.*

PITTSBURGH, PA.

THE increased urinary sodium excretion that results from the administration of ACTH to patients with virilizing congenital adrenal hyperplasia was first reported in 1949 by Dr. Wilkins in association with Roger A. Lewis.[1] The following year these observations were extended to include patients with the salt-losing variety of the syndrome.[2] It was noted that the potassium excretion of different patients responded in varying fashion to ACTH, but there was a consistent sodium diuresis on the day ACTH was given. The suggestion was made that patients with congenital adrenal hyperplasia associated with Addisonian crises might secrete adrenal steroids which promote sodium excretion rather than sodium retention. In 1951 work was reported from the same laboratory demonstrating increased sodium excretion in response to ACTH administration in newborn infants. These infants were able to respond to desoxycorticosterone administration by retaining sodium.[3, 4] At this time it was suggested that there might be either secretion of a hormone not present in later life, or an alteration in the balance between hormones having varying quantitative or qualitative effects on sodium excretion. The latter alternative was considered on the basis of the theory advanced by Thorn and his associates[5] suggesting that weak sodium retainers, such as Compounds E and F, compete with potent sodium retainers, such as desoxycorticosterone and aldosterone, and thereby lessen sodium retention. However, as knowledge increased, this latter possibility became quite improbable. Further stimulus to the theory that patients with adrenal hyperplasia excreted a salt-losing hormone was provided by the work of Prader, Spahr, and Neher.[6] These workers reported increased aldosterone excretion in patients with congenital adrenal hyperplasia who had no clinical salt-losing symptoms, or whose salt-losing symptoms had disappeared with age. Normal or slightly increased excretion of aldosterone was demonstrated in infants with active salt-losing symptoms associated with congenital adrenal hyperplasia. More recently Blizzard, Liddle, Migeon, and Wilkins[7] reported that, although infants with the salt-losing syndrome associated with adrenal hyperplasia did excrete aldosterone, the quantity of excretion

*From the Department of Pediatrics, University of Pittsburgh Medical School and Children's Hospital of Pittsburgh.*

*Much of the work in this study in the author's laboratory was supported by grants from the American Cancer Society, Inc., Martha O. Freas Memorial MOF-2, and the National Institutes of Health, Public Health Service, Grant A-1103(C) and Grant A-293(C).*

**Address, The University of Pittsburgh School of Medicine, Pittsburgh 13, Pa.*

was similar to that of normal infants who were receiving adequate intake of salt and who had no stimulus for the increased production of aldosterone. Patients with adrenal hyperplasia who were not salt losers excreted increased amounts of aldosterone. They further pointed out that salt restriction did not increase the excretion of aldosterone by patients with the salt-losing syndrome. It should be noted that Luetscher[8] had demonstrated normal excretion of aldosterone in newborn infants. The same infants studied in our laboratory responded to ACTH administration with increased excretion of sodium in the urine. Eberlein and Bongiovanni[9] observed a correlation between the amount of tetrahydrocortisone excreted and clinical salt-losing tendencies. The patients with adrenal hyperplasia and no salt-losing tendency excreted the largest amount of tetrahydrocortisone; active "salt losers" excreted a minimal amount; and those patients who had salt loss under stress only excreted less than normal amounts, but more than that excreted by active "salt losers." They concluded that the important difference between the salt-losing patient and the one with adrenal hyperplasia without disturbed electrolyte regulation seemed to be the relative amount of hydrocortisone secreted by the hyperplastic adrenals. They suggest that small amounts of hydrocortisone are an essential prerequisite for the action of aldosterone. The possibility has been investigated directly by Ross.[10] His results do not rule it out entirely, but certainly do not support it.

Colle[11] and others at the University of Minnesota have demonstrated the lack of sodium retention and at times actual sodium loss postoperatively in newborn infants.

## ARGUMENT

There are three major hypotheses for the salt-losing tendency of patients with adrenal hyperplasia:

1. Deficient excretion of the normal salt-retaining steroid, aldosterone.

2. Deficient secretion of hydrocortisone, the normal glucocorticoid, which has relatively less, but clinically significant salt-retaining properties.

3. The presence of factors promoting the urinary excretion of salt.

Absence of secretion of aldosterone by newborn infants and patients with congenital adrenal hyperplasia has been ruled out by the work of Luetscher[8] and that of Prader.[6]

The report from Wilkins'[7] laboratory suggests that normal newborn infants, as well as infants with salt-losing congenital adrenal hyperplasia, have some limitation in their ability to increase secretion of aldosterone in response to restriction of dietary sodium. This report also points out that the limitation in secretion of aldosterone continues even into that later period in life when the patient no longer has symptoms of salt loss. The latter observation is discordant with the findings of Prader.[6] The evidence regarding secretion of hydrocortisone is also inconsistent. It is well known that children with adrenal hyperplasia secrete less than normal quantities of hydrocortisone and the evidence suggests that patients with the salt-losing variety of the syndrome secrete even less than do the non–salt losers.[9] On the other hand, normal infants after the first week or two of life effectively increase their secretion of hydrocortisone in response to ACTH at the same time that they are having a sodium diuresis.[3, 4] Even if the limitations in the secretion of aldosterone and hydrocortisone were unequivocal, the sodium diuresis which follows administration of ACTH would not be explained.

A tenable explanation for the findings of Eberlein and Bongiovanni[9] is that patients with congenital adrenal hyperplasia who are not salt losers are the less severely affected individuals, presumably with a less complete block of 21 hydroxylation. They, therefore, produce more hydrocortisone as well as aldosterone. It is conceivable also that the production of a salt-excreting factor might be inversely related to the production of hydrocortisone and aldosterone.

Patients with the non–salt-losing type of congenital adrenal hyperplasia actually se-

crete an excessive amount of aldosterone and respond to dietary restriction of sodium with even greater secretion. These patients secrete greater amounts of hydrocortisone than do the salt losers. However, non–salt losers, too, respond to ACTH with sodium diuresis.[1, 6, 7, 9] The existence of an antagonist to aldosterone is further suggested by the fact that patients secreting abnormally large quantities of aldosterone never demonstrate any clinical stigmas of hyperaldosteronism.

The existence of a salt-losing component is implied by the amounts of desoxycorticosterone and salt required to treat an infant with the salt-losing form of adrenal hyperplasia. If he is not given cortisone, he requires huge doses of these materials; more, in fact, than one would expect the patient who had had a complete adrenalectomy would need. In spite of this therapy, his serum potassium concentration does not usually return all the way to the normal range. When such a patient is given hydrocortisone, or one of its analogues, which inhibits his own adrenal secretion, he requires much less desoxycorticosterone for maintenance and the concentration of his serum potassium becomes normal. Indeed, it is advisable to treat infants who are salt losers with hydrocortisone before or simultaneously with administration of desoxycorticosterone. If one waits until the condition has been fairly well controlled with desoxycorticosterone and then administers the glucocorticoid, there is a danger of producing hypernatremia. Thus, this situation also suggests that the antagonist to aldosterone disappears when ACTH secretion is suppressed.

I chose to interpret the response of the newborn to surgery reported by Colle[11] and by Rickham[12] as being consistent with the existence of a salt-losing hormone. However, these findings were variable, as one would expect, and may be interpreted in many other ways.

We have worked since 1951 on the hypothesis that at least newborn infants and patients with congenital adrenal hyperplasia secrete a material that has a diuretic action for sodium. Since the sodium diuresis occurs after administration of ACTH and occurs in patients with adrenal disease, it was assumed that the effective substance is of adrenal origin.*

It is possible that there may not be a salt-losing hormone, per se, but that the loss of sodium is the result of the aggregate effect of many nonspecific materials. Thus, in certain clinical situations where secretion of aldosterone and of hydrocortisone is limited, the stimulated adrenals could secrete a group of inert or extremely weak salt-retaining substances, which would compete with aldosterone and hydrocortisone for cellular sites of activity. Under these circumstances, akin to the experimental work of Thorn with desoxycorticosterone and Compound E, sodium excretion would increase until, for practical purposes, the entire effect of the aldosterone had been eliminated. After that, excretion of sodium should remain at a constant level. The only results that bear on this point are those obtained from prolonged administration of ACTH to newborn infants.[4, 13] Progressively greater sodium loses were observed for a few weeks.[4] Eventually a plateau of excretion was reached, followed soon thereafter by actual retention of sodium.[13] This last suggests that the plateau of excretion may not have resulted from the maximum inhibition of aldosterone activity, but rather from a changing pattern of steroid secretion.

Finally, if a salt-losing hormone exists, several questions are of immediate interest. Is the hormone secreted only by the adrenals of newborn infants and of individuals with certain diseases? Or do normal adrenals secrete minute amounts of a salt-losing hormone whose effect is obscured by other hormones? Second, does this postulated material act directly upon the renal tubular cells to promote diuresis of sodium or is it a competitive inhibitor of aldosterone? Neither question can be answered, although some evidence relevant to the first question

*There is the possibility that no abnormal hormone is actually secreted, but that normal hormones are metabolized in an abnormal fashion.

will be presented later. The only data concerning the question of competitive inhibition or direct renal tubular action are not very helpful. The groups of patients who respond to ACTH with an abnormal diuresis of sodium have all produced aldosterone and hydrocortisone. The synthetic materials, such as the spirolactones of Kagawa,[14] act as competitive inhibitors of aldosterone. The relevance of either observation is questionable, and the spirolactone is inactive in tests for salt-losing hormone to be described later.

## RECENT AND CURRENT WORK

Many attempts have been made to prove the hypothesis that the adrenal cortex, stimulated by ACTH, secretes a sodium-excreting factor. Each group of workers who has attempted this had proposed first to isolate a material with a sodium-excreting effect demonstrable in some form of bioassay system, to identify this material, and finally to prove that it has the same effect in humans. Two groups of workers, Neher, Meystre and Wettstein[15] in Switzerland and our own group[16] in this country, have obtained from human urines material causing diuresis of sodium when injected into test animals. Others have not been successful in this attempt. No one has successfully identified the material or demonstrated its effectiveness in humans.

Since any attempt to find a salt-losing hormone must involve preliminary identification by bioassay, it would be wise to discuss briefly methods of bioassay at this point. One must remember that material causing a rat to increase his excretion of urinary sodium might have no physiologic significance. Most workers have attempted to bioassay salt-losing materials in adrenalectomized animals while they were receiving standard amounts of desoxycorticosterone or aldosterone. These methods have been successful in demonstrating the antagonism to aldosterone of the spirolactones, etc. By these methods, however, hormones with a weak salt-retaining effect and hormones increasing glomerular filtration have, at times, been shown to lead to increased excretion of sodium.[8, 17] We have carried out bioassays in intact rats. This obviously has the disadvantage of variations produced by the rat's own adrenal secretions. It does have the advantage of minimizing the possibility of an effect being mediated through increased glomerular filtration produced by the steroid. Creatinine excretions are employed as a reference in order to check this point and as an index for completeness in urinary collections. It is conceivable that Gunn rats would be responsive bioassay animals.

The Swiss groups have called the material producing diuresis of sodium in rats Sodium Excreting Factor (SEF). We shall use this nomenclature since it seems probable that it is the same material we had reported, and its hormonal identity has not been proved. Neher and his co-workers found this material in urine of patients with congenital adrenal hyperplasia, of normal adults following surgery, and of newborn infants. We have obtained material with sodium-excreting activity from the urine of newborn infants given ACTH, of patients with congenital adrenal hyperplasia, and of patients with hyperkalemic familial periodic paralysis. In addition, we have obtained it from one healthy adult under circumstances to be mentioned later. These urines were extracted with an organic solvent before and after enzymatic and acid hydrolysis, and then more or less isolated by a series of manipulations involving column and paper chromatography, differential solubility at different pH's, etc. While the methods employed by the two groups were slightly different, the active fractions behaved similarly when run on the same paper chromatography solvent systems. Fig. 1 is a schematic presentation of methods of extraction and purification of urinary extracts as carried out in our laboratory. Neher used adrenalectomized rats for bioassay; we used intact rats. Combinations of the 3 $\alpha$ and the 3 $\beta$, 16 $\alpha$ dihydroxy pregnane 20-one, 3 $\alpha$ and 3 $\beta$, 16 $\alpha$ dihydroxy allo pregnane 20-one were identified in the active fraction by

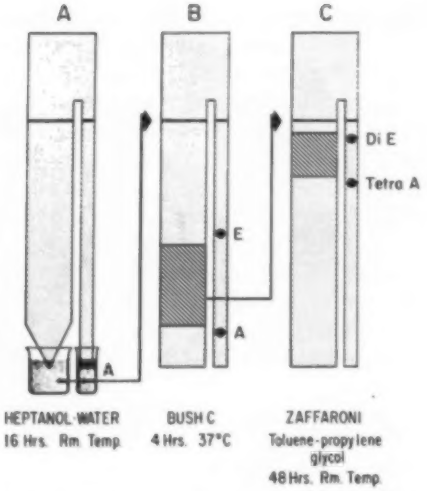



**Fig. 1.** Schema for isolation of SEF employed in author's laboratory:

1. Urine is extracted with 2 volumes of redistilled $CH_2Cl_2$; residue is hydrolized with glucuronidase and with acid and extracted again with $CH_2Cl_2$.
2. Extracts may be pooled at this point or run separately. $CH_2Cl_2$ extracts are washed with 0.1 N NaOH and then with distilled $H_2O$.
3. Water soluble material is discarded and methylene chloride containing steroid material is dried in an air blast while being maintained at 30° C.
4. The dried extract is taken up in absolute ethanol and applied to origin line of paper to be chromatographed for 16 hours in the heptanol water system, or alternatively is taken up in methylene chloride and applied to Florisil column. The column is washed with methylene chloride and then with 1 per cent ethanol in methylene chloride. The material is then eluted with 25 per cent ethanol in methylene chloride.
5. The water run off from the above chromatogram is extracted with methylene chloride, or the Florisil column eluate is dried.
6. Methylene chloride extract is dried, taken up in absolute ethanol, and applied to paper to be chromatographed for 4 hours in system *C* of *Bush*.
7. Area of Bush chromatogram from just below the standard *E* to just above the standard *A* in the parallel strip is cut out and eluted with absolute ethanol.
8. Ethanol eluate is partially evaporated and the remainder is applied to origin of propylene glycol saturated paper to be chromatographed for 48 hours in the *toluene-propylene glycol* system of *Zaffaroni*.
9. Area of Zaffaroni chromatograph from standard *Di E* to *Tetra A* is cut out and eluted for SEF.

Neher, Meystre, and Wettstein.[15] They felt that one or more of these compounds were active sodium-excreting factors; the allo pregnane material was also isolated from hog adrenals. These workers synthesized the substances. The suggestion that SEF was one of the 3 $\beta$, 16 $\alpha$ dihydroxy 20-keto pregnanes received further support from the demonstration by Bernstein[18] that susbstitution of hydroxyl groups in the 16 position of certain corticoid molecules markedly reduced their sodium-retaining properties. Triamcinolone has irregularly produced diuresis of sodium when given to intact rats in our laboratory. Unfortunately synthetic 3, 16 dihydroxy 20-keto pregnanes did not show clear-cut sodium diuretic effect when injected in humans.[19] Neher and Wettstein were kind enough to send us some of their synthetic materials. We could not produce a diuresis of sodium in intact rats by injection of these materials. In addition, the Swiss workers found that the most biologically active extract obtainable from the urine of newborn infants was in the free or unhydrolyzed fraction, whereas the greatest quantity of the 3, 16 dihydroxy 20-keto pregnanes was in the fraction obtained after hydrolysis.[16]

Our experience has been somewhat similar. Sodium diuretic activity has been obtained from a chromatographic fraction of urines in which the 3, 16 dihydroxy 20-keto pregnanes are located. Infra-red analysis of these fractions has shown patterns suggestive of mixtures of these materials. The biologic activity is lost in the process of further purification, but the infra-red pattern does not change. In addition to finding a salt-excreting fraction in the urine of patients with congenital adrenal hyperplasia and of newborn infants given ACTH, we[20] have found similar material in the urine of patients with hyperkalemic familial periodic paralysis. We, too, found that biologic activity was, in the urine of newborn infants, concentrated in the free or unhydrolyzed fraction. In patients with hyperkalemic familial periodic paralysis the activity seemed to be more concentrated in the fraction obtained after hydrolysis. However,

observations on this point are somewhat sketchy. One would expect to find much activity in the free fraction in newborn infants since they do not conjugate steroids as well as older children and excrete a considerable part of their total steroids as free steroid. We have been able to reproduce the biologic effect of an extract on a subsequent assay only when no manipulation of the extract was carried out between assays. Whenever we have tried to purify the material further and then carry out a bioassay, all activity has been lost.

When ACTH was given to a patient with hyperkalemic familial periodic paralysis, his urinary sodium decreased and his urinary potassium increased markedly. Yet he and other patients with the same disease excrete increased amounts of biologically active SEF when given ACTH. The same situation pertains to the adult patients from whom Neher was able to obtain SEF in urine collected postoperatively. There was no diuresis of sodium accompanying this. Presumably both these groups of patients were able to secrete enough salt-retaining hormones in response to ACTH and surgery, respectively, to more than compensate for any increased excretion of SEF. If this be true, it would suggest that normal individuals excrete SEF for which they are easily able to compensate. It would further imply that our failure to demonstrate this material in the urine of healthy individuals resulted from lack of sensitivity of our methods and that we have demonstrated SEF only when it exists in abnormal quantities.

Pooled urine from full-term newborn infants unstimulated with ACTH did not contain demonstrable quantities of SEF. We have not extracted urine of normal adults after operation, nor have we used volumes as large as those extracted by the Swiss group. We have been unable to obtain SEF from the urine of older children with or without ACTH stimulation, however. The children were receiving ACTH for various therapeutic reasons, but demonstrated no evidence of adrenal disease. The children from whom urine was collected without ACTH stimulation were apparently normal individuals recovering from acute illnesses.

SEF was found in the urine of one apparently healthy male adult. This individual had conditioned himself to ingest enormous quantities of fluid and salt as a young boy. It was estimated that he ingested at least 15 Gm. of added salt each day over and

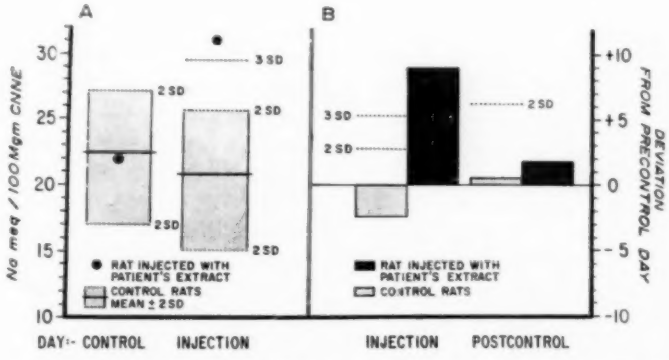



**Fig. 2.** Sodium diuretic effect in rats of extract of urine from patient chronically ingesting excessive salt.

*A* and *B* are results from one experiment expressed as follows:

*A* compares sodium excretion on the precontrol day with that on the day of injection.

*B* depicts change in sodium excretion from the precontrol day to the day of injection and to the postcontrol day.

SD = Standard deviation.

**Table I.** Assay of SEF in urine of patients with adrenal hyperplasia, hyperkalemic familial periodic paralysis, newborn premature and full term infants and in one adult with chronically increased salt ingestion.

Assays were carried out in intact male white rats. The significance of figures refer to the differences in excretion of sodium.

| *Animals* | *No.* | *Source of active fraction* | *Na (mEq./100 mg. creatinine)* | *Na/K* | *Creatinine mg./day* | *Significance* |
|---|---|---|---|---|---|---|
| Controls* | 69 | | 19.9 ± 1.00 (SEM) | .46 | 6.4 | $p < .01$ |
| Test | 10 | Patients with hyperkalemic familial periodic paralysis | 30.0 ± 0.61 | .67 | 6.7 | |
| Controls | 137 | | 19.0 ± 1.24 | .47 | 6.0 | $p < .01$ |
| Test | 26 | Premature newborns given ACTH | (13) 32.5 ± 2.29 | .73 | | |
| | | | (13) 18.8 ± 0.91 | .42 | | |
| | | | (26) 25.7 ± 1.81 | .58 | 6.2 | |
| Controls | 55 | | 19.1 ± 1.40 | .45 | 8.1 | $p < .01$ |
| Test | 5 | Patients with adrenal hyperplasia | 32.2 ± 5.37 | .73 | 7.0 | |
| Controls | 22 | | 19.2 | .58 | 5.1 | |
| Test | 4 | Full-term newborns | 20.0 | .63 | 5.4 | |
| Controls | 14 | | 22.4 ± 2.07 (SD) | .51 | 6.1 | $p < .01$ |
| Test | 1 | Adult with extremely large salt intake | 31 | .66 | 6.4 | |

*Control rats were injected with olive oil or inert urinary extracts.

above the natural salt content of his food and that added in cooking. Fig. 2 portrays the results with an extract from a single urine collection from this adult with chronic excessive ingestion of salt. The data are graphed in two ways. *A* represents 24-hour sodium output in control rats on the day before and on the day of injection of inert materials, compared to excretion of a single rat before and after injection of active extract. *B* represents the changes in excretion of sodium by control and test rats from the day prior to injection and the day of injection to the postcontrol day. Either method of calculation demonstrates that the effect of diuresis of sodium produced by the extract was statistically highly significant. In view of this observation two physicians ingested 15 Gm. of salt per day for 3 days over and above that in their ordinary diet. One of them became edematous on the second day, but began to lose the edema on the third day. The urinary excretion of sodium of both quadrupled. In spite of this, extracts from 4 day pools of urine from each subject contained no demonstrable SEF. It is not clear whether or not these failures resulted from inadequacy of the methods of analysis or whether the salt ingestion was not of sufficient magnitude and for a long enough period of time.

Table I presents the data from the bioassays of SEF from different groups of patients. The daily excretion of creatinine by the assay rats varied from 5 to 8 mg. per day. There was no consistent change in creatinine excretion by the rats given SEF-containing extracts. There was no significant change in potassium excretion at the time of diuresis of sodium. One half of the extracts from urines of newborn infants were inactive. This explains the lower mean sodium excretion for their group than for the other two groups. The possible reasons for the failure to demonstrate SEF activity more consistently have been discussed elsewhere.[17]

Considerable data have been accumulated concerning the characteristics of SEF. However, since the infra-red patterns of this

fraction have been shown to represent material other than sodium-excreting material, so much doubt must be cast upon the reliability of the evidence concerning staining qualities, ethanol and sulfuric acid absorption peaks in the ultra-violet, etc., that these should not be reported at the present time.

The only information available at the present time upon which we may rely concerns the behavior of SEF in column and paper chromatography. The active material is eluted from a Florisil column in the 1 to 10 per cent ethanol in chloroform fraction. As shown in the schema it is elutable from the paper run in the Bush system C in the fraction from the area below standard Compound E to that above standard Compound A. Then when it is chromatographed in the toluene-propylene glycol system of Zaffaroni it is found in the area from dihydro E to tetrahydro A. This behavior is not described more precisely because it was only after we had been employing these systems for several years that we awakened to the fact that the Rf in these systems (most notably in the Zaffaroni) was influenced by the quantity of other steroids and perhaps of nonspecific material placed upon the paper.

## CONCLUSION

I believe that it is impossible to explain a diuresis of sodium following administration of ACTH by any deficiency in secretion of aldosterone and/or of hydrocortisone per se. Therefore, I believe it must be concluded that this diuresis is produced by a congeries of inert materials competitively inhibiting aldosterone or by an actual salt-losing hormone that may or may not act in the same manner. The limitation in ability to increase aldosterone secretion that apparently exists in newborn infants and certain patients with adrenal hyperplasia would tend to allow diuresis of sodium when either of these two mechanisms is operative. In at least one instance, however, diuresis of sodium follows administration of ACTH to individuals with greater than normal aldosterone-secreting abilities, i.e., patients with non–salt-losing congenital adrenal hyperplasia.

I cannot properly evaluate the role of the SEF material extracted from urine. The 3, 16 dihydroxy 20-keto pregnanes which have been in the fractions employed both in our experiments and in those of Neher and associates do not produce diuresis of sodium in our intact rats nor did Neher find any loss of salt when they were administered to man. Three possibilities come readily to mind: (1) diuresis of sodium in rats injected with SEF from extracts of urine is at best a nonspecific effect of a urinary fraction containing no salt-retaining hormones; (2) one or several of the 3, 16 dihydroxy 20-keto pregnanes has a weak salt-excreting effect that is compensated for by some unknown factor in the intact rat and in the human; or (3) SEF is a very potent material which accompanies the 3, 16 dihydroxy 20-keto pregnanes. If the last is correct the concentration of SEF is so small that it gives no recognizable interference with the infra-red patterns of the 3, 16 dihydroxy pregnanes. If this last possibility be true, the SEF may be only one of many which was uncovered by a random combination of suitable factors in our experiments. It might have no human physiologic significance. Final definition awaits more experimentation.

Since this article was written, Rosemberg and co-workers[21] have reported detection of a similar salt-losing material in the urine of a patient with congenital adrenal hyperplasia. Although they too have not been able to identify the substance, their work does lend confirmation to the observations of Neher[15] and of our group.

## REFERENCES

1. Lewis, R. A., and Wilkins, L.: The Effect of Adrenocorticotrophic Hormone in Congenital Adrenal Hyperplasia With Virilism and in Cushing's Syndrome, Treated With Methyl Testosterone, J. Clin. Invest. **28:** 394, 1949.
2. Wilkins, L., Klein, R., and Lewis, R. A.: The Response to ACTH in Various Types of Adrenal Hyperplasia, Proc. 1st Clin. ACTH Conf., Philadelphia, 1950, The Blakiston Company, p. 184.
3. Klein, R.: Some Aspects of Adrenal Physi-

ology in the Newborn Period and Early Infancy, Proc. 2nd Clin. ACTH Conf., Philadelphia, 1951, The Blakiston Company, vol. 1, p. 123.
4. Klein, R.: Adrenocortical Control of Sodium and Potassium Excretion in the Newborn Period, J. Clin. Invest. **30:** 318, 1951.
5. Thorn, G. W., Forsham, P. H., Bennett, L. I., Roche, M., Reiss, R. S., Slessor, A., Flink, E. B., and Somerville, W.: Clinical and Metabolic Changes in Addison's Disease Following the Administration of Compound E Acetate, Tr. A. Am. Physicians **62:** 233, 1949.
6. Prader, A., Spahr, A., and Neher, R.: Erhöte Aldosteronausschedung beim kongentalen adrenogenitalen Syndrom, Schweiz. med. Wchnschr. **85:** 1085, 1955.
7. Blizzard, R. M., Liddle, G. W., Migeon, C. J., and Wilkins, L.: Aldosterone Excretion in Virilizing Adrenal Hyperplasia, J. Clin. Invest. **38:** 1442, 1959.
8. Luetscher, J. A., Jr.: Studies of Aldosterone in Relation to Water and Electrolyte Balance in Man, Recent Progr. Hormone Research **12:** 175, 1956.
9. Eberlein, W. R.: The Salt-Losing Form of Congenital Adrenal Hyperplasia, Pediatrics **21:** 667, 1958.
10. Ross, E. J.: Modification of Effects of Aldosterone on Electrolytes by Simultaneous Administration of Corticosterone and Hydrocortisone, J. Clin. Endocrinol. **20:** 229, 1960.
11. Colle, Eleanor, and Paulsen, Elsa P.: Response of the Newborn Infant to Major Surgery, Pediatrics **23:** 1063, 1959.
12. Rickham, P. P.: Metabolic Response to Neonatal Surgery, Cambridge, 1957, Harvard University Press.
13. Lanman, J. T.: Adrenal Function in Premature Infants, Pediatrics **12:** 62, 1953.
14. Kagawa, C. M., Cella, J. A., and VanArman, C. G.: Action of New Steroids in Blocking Effects of Aldosterone and Desoxycorticosterone on Salt, Science **126:** 1015, 1957.
15. Neher, R., Meystre, Ch., and Wettstein, A.: Neue 16 α Hydroxysteroide aus menschlichen Urin und aus schweinenebennieren Isolierung, Konstitution, Synthesen, Helvet. chim. acta **42:** 32, 1958.
16. Klein, R., Taylor, P., Papadatos, C., Laron, C., Keele, D., Fortunato, J., Byers, C., and Billings, C.: Sodium Losing Material in Human Urine, Proc. Soc. Exper. Biol. & Med. **98:** 863, 1958.
17. Kagawa, C. M., and VanArman, C. G.: Effects of Cortisone and Hydrocortisone on Sodium Excretion in Adrenalectomized Rats, Proc. Soc. Exper. Biol. & Med. **94:** 683, 1957.
18. Bernstein, S.: The Chemistry and Biological Activities of 16 Hydroxylated Steroids, Recent Progr. Hormone Research **15:** 1, 1958.
19. Neher, R.: Personal communication.
20. Klein, R., and Egan, T.: Changes in Sodium, Potassium, and Water in Patients With Hyperkalemic Familial Periodic Paralysis (in press).
21. Rosemberg, E., DuFault, F. X., Jr., Bloch, E., Budnitz, E., Butler, P., and Brem, J.: Effects of Progressive Reduction of Sodium Intake on Steroid Excretion and Electrolyte Balance in Salt-Losing Adrenal Hyperplasia, J. Clin. Endocrinol. **20:** 214, 1960.

# Two new subvariants of virilizing adrenal hyperplasia

José Cara, M.D.,* and Lytt I. Gardner, M.D.**

SYRACUSE, N. Y.

UNDER the designation of virilizing adrenal hyperplasia the pediatrician may face several different clinical pictures. Each of these subvariants, however, shares in common the androgenic effects of the hyperplastic adrenals and the increased urinary excretion of 17-ketosteroids. In addition to this picture of virilization there may be other symptoms and signs which delineate a series of types of this basic syndrome. It is understandable that the early clinical descriptions of virilizing adrenal hyperplasia were concerned mostly with the abnormal or precocious sexual development of the patients, since this was the more obvious manifestation. Furthermore, many patients with excessive sodium loss very likely died before the clinical manifestations of excessive androgen production were identified and thus these manifestations were not diagnosed.

*From the Department of Pediatrics, State University of New York, Upstate Medical Center, Syracuse, N. Y.*

*This study was supported in part by Research Grant A-2504 (C1) and Training Grant 2G-346, National Institutes of Health, United States Public Health Service.*

*Presented in part at the IX International Congress of Paediatrics, Montreal, Canada, July 19 to 25, 1959.*

**Senior postdoctoral trainee, Division of General Medical Sciences, National Institutes of Health.*

***Address, Department of Pediatrics, State University of New York, Upstate Medical Center, 766 Irving Avenue, Syracuse 10, N. Y.*

In 1939 Butler and colleagues[1] and in 1940 Wilkins and associates[2] called attention to some manifestations of excessive sodium loss in 2 boys with virilizing adrenal hyperplasia. Over the succeeding 20 years there have followed numerous reports describing other characteristics coexistent with virilization. It was found that in two cases hypoglycemia was an important part of the picture.[3, 4] Arterial hypertension was described in several patients.[5, 6] It was also seen that although the virilizing hyperplasia was in most cases a congenital condition, certain few patients developed the disease postnatally.[7] In rare instances it was noted that patients had adolescent development even without corticosteroid treatment.[8, 9] In the majority of cases there was no puberal development unless corticosteroid treatment was provided.[10]

The present study concerns two additional clinical subvariants: (1) virilizing adrenal hyperplasia with "late" sodium loss and (2) virilizing adrenal hyperplasia with periodic fever. In the first instance there was development of excessive sodium loss in a female pseudohermaphrodite at 14 months of age. Up to the age of the episode she had shown no obvious symptoms or signs referable to overt sodium loss. The other subvariant, exemplified by the second case

history, is a boy with congenital adrenal hyperplasia and episodes of periodic fever with other symptoms and signs apparently related to the presence of a 17-ketosteroid not normally found in plasma.[11, 12]

1. **Virilizing adrenal hyperplasia with late sodium loss.** P. G., a girl, was referred to the Upstate Medical Center at the age of 5 months for "undetermined sex and failure to gain properly." A brother and a sister, 8 and 6 years older, respectively, than the patient, were living and well. The maternal grandmother had a stillborn child who was, according to the physician who attended her, "neither" [boy or girl], when he replied to her question as to gender of the infant. No other information could be obtained about this infant. There was no other history of endocrine or metabolic diseases. The child breathed and cried spontaneously at the time of birth; her weight was 4,000 grams. She "vomited" during the newborn period, according to the mother, but there was no history of circulatory collapse. At about 2 weeks of age she was given a formula of evaporated milk and Karo syrup. On this regimen her weight increased from 4,000 to 5,550 grams by 5 months of age (Fig. 1).

The patient had been considered to be a male with hypospadias and cryptorchidism at birth and was given a male name. An examination at the age of 6 weeks revealed the absence of testes and evoked the problem of undetermined sex. She was said to have fed poorly at home where she was cared for with self-demand feeding by the mother. There had been no episodes of overt collapse or dehydration. There was no history of infectious diseases.

On physical examination at age 5 months she was well nourished, well developed, and in no distress. She was alert, active, and had a quick responsiveness to people around her, and she cooed, smiled, and cried as the situation was appropriate. She could lift her head and arms and could maintain a sitting position if propped. She was afebrile and her skin was of good color. Her measurements were as follows: crown-heel, 59.5 cm.; crown-rump, 40.5 cm.; head, 40.5 cm.; and chest, 40.1 cm. There was an enlarged clitoris of about 1.5 cm. in length and a urogenital sinus. There were no palpable gonads. Rectal examination gave the impression of a palpable uterus. Sex-chromatin studies on smears of the buccal mucosa revealed a positive pattern. The urinary excretion of 17-ketosteroids ranged between 5.1 and 7.7 mg. per 24 hours and fell to a value of 3.9 mg. per 24 hours after 3 days of cortisone treatment. While the infant was hospitalized her weight decreased from 5,550 to 5,270 grams despite careful nursing attention and self-demand feeding. She was sent home on oral hydrocortisone therapy in the form of F-Cortef, 10 mg. per day in 4 divided doses. Shortly after this admission the rate of gain in weight was noted to have slackened (Fig. 2). By 12 months of age her general appearance was poor and her weight was below the third percentile by then. At the age of 14 months she was admitted to another hospital with a history of vomiting for the previous 5 days. She weighed 6,450 grams contrasted with a weight of 7,250 grams recorded 2

**Fig. 1.** Photograph of P. G. at 5 months of age showing the abnormal external genitalia. No treatment had been started, and no signs of excessive sodium loss were yet evident.

weeks before and appeared to be dehydrated. Serum chloride was 124 mEq. per liter; carbon dioxide, 10 mEq. per liter; potassium, 3 mEq. per liter; and serum sodium, 144 mEq. per liter. She was treated with intravenous electrolyte solutions for the first 48 hours of hospitalization and then with fluids by mouth. Her weight rose to 7,100 grams, but 1 week later it had gradually dropped to 6,600 grams; she remained at this level for 3 or 4 days thereafter. On the eighth hospital day the serum sodium was 126 mEq. per liter; potassium, 5.6 mEq. per liter; chloride, 87 mEq. per liter; and carbon dioxide, 23.7 mEq. per liter. She was immediately started on 5 Gm. of sodium chloride daily, which was added to her formula. This was maintained for the next month with very little effect on the gain in weight or on the serum sodium concentration. At the age of 15 months she was started on desoxycorticosterone trimethylacetate intramuscularly. She was given an initial dose of 12.5 mg. after which she had a marked gain in weight and an increase in appetite and in vitality. Four weeks after this administration, a second dose with 25 mg. was given, and again her weight increased steadily during the following 3 weeks. The fourth week after the second dose, her weight tended to become stationary. The administration of 37.5 mg. at this time was followed by a resumption of gain in weight. Although the weight increment was impressive and the mother felt that the child was doing better than ever, the desoxycorticosterone trimethylacetate in this dosage apparently had not been sufficient to raise the serum sodium concentration (Fig. 2). At this time the urinary excretion of 17-ketosteroids on 2 successive days was 5.5 mg. and 3.3 mg., respectively. The dose of hydrocortisone was increased to 15 mg. daily per mouth and the desoxycorticosterone trimethylacetate to 50 mg. intramuscularly at monthly intervals. After the second dose of 50 mg. of desoxycorticosterone trimethylacetate the values of serum sodium were consistently normal. By the following year she was doing well except for occasional

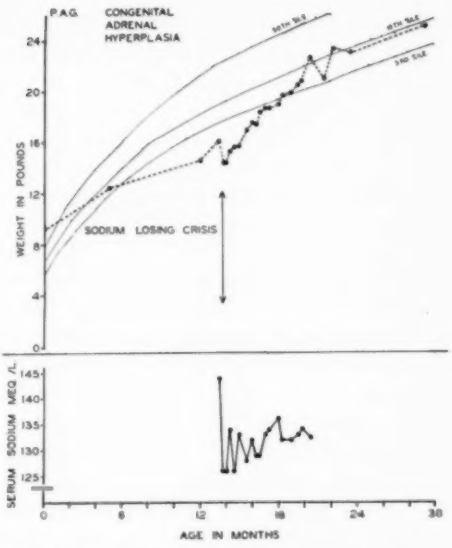



**Fig. 2.** Chart showing the weight curve of P. G. and the sodium-losing crisis at 14 months of age. The rate of gain in weight increased sharply immediately after specific treatment with desoxycorticosterone trimethylacetate was provided. Serum sodium only reached normal values when a dosage of 50 mg. desoxycorticosterone trimethylacetate monthly was attained at about 22 months of age.

episodes of fever lasting 2 to 3 days. No changes in the doses of hydrocortisone or desoxycorticosterone trimethylacetate were found to be necessary. The added salt was discontinued when the patient was 2 years of age, although her mother continued to salt her food heavily. She was seen again at the age of 2½ years. At that time she was very active, alert, and healthy looking, though very small for her age. The height-age, weight-age, and bone-age ratios were about equal at 1 year below her chronologic age. Serum chloride was 110 mEq. per liter; sodium, 141 mEq. per liter; and potassium, 4.3 mEq. per liter. At about 3 years of age she had 3 episodes of fever which required hospitalization for 2 or 3 days and which on one occasion required intravenous fluids because of circulatory collapse and low serum sodium values. After the acute episode, however, it was not found necessary to increase the dose of desoxycorticosterone trimethylacetate.

In summary, this girl with virilizing adrenal hyperplasia has been receiving corticosteroid treatment since the age of 6 months. At the age of 14 months she went into a severe sodium-losing crisis, without having had a previous history of such episodes, and has received desoxycorticosterone trimethylacetate therapy intramuscularly since that time.

*Comment.* Children with overt episodes of sodium loss represent about one fourth of the cases of virilizing adrenal hyperplasia. The usual descriptions of this clinical form emphasize, as a rule, the early beginning of the symptoms and signs of excessive sodium loss.[7, 13, 14] A review of 109 cases in which the time of onset of the initial symptoms could be stated revealed that in 60 cases (44 per cent) the initial symptoms began during the first week of life and in all cases of this series before the age of 7 weeks.[14] Wilkins and others[2] reported a boy with virilizing adrenal hyperplasia who at the age of 1 year developed marked craving for salt and salted foods. There was no reference to previous symptoms. When the child was hospitalized at 3 7/12 years of age and could not get the amount of salt he used to have at home, he died suddenly. Postmortem blood obtained by cardiac puncture revealed a serum sodium value of 111 mEq. per liter.

In view of these observations, it would now seem unwise to exclude the possibility of overt sodium loss at a later date in patients who had had no such crises in the first weeks of life.

2. **Virilizing adrenal hyperplasia with periodic fever.** D. C. (SMH No. 103781), a white boy, was first seen at the Upstate Medical Center at 7 9/12 years of age with a known history of congenital adrenal hyperplasia for the 2 previous years. The parents were both living and well. One brother, 3 years younger, was living and well. There was no family history of unusual body growth, sexual abnormalities, or other endocrine disorders. Pregnancy had been uneventful, with no special medication during that time. The birth was normal, full term, and spontaneous with a vertex presentation. Birth weight was 3,300 grams. Neonatal course was normal. On several instances during the early years of life the boy had episodes of fever with sweating, collapse, and conjunctival injection.

The patient had had German measles, chickenpox, mumps, whooping cough, and on two occasions a diagnosis of "virus pneumonia" was made. He began school at 5 years of age, has always been a good student, and has received excellent marks.

At 1½ years of age it was noted that the child had erections and that the penis was

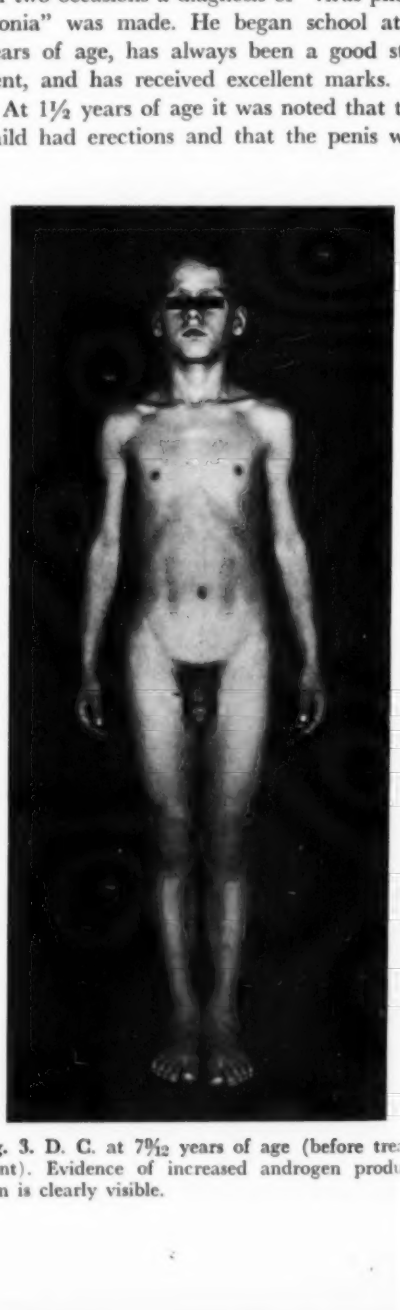


**Fig. 3. D. C. at 7 9/12 years of age (before treatment). Evidence of increased androgen production is clearly visible.**

large. However, the significance of this was not appreciated until the age of 5 years when he entered school. At this time the school physician noted growth of pubic hair, an enlarged penis, and acne. The child was then studied in another hospital where the diagnosis of virilizing adrenal hyperplasia was made. The urinary 17-ketosteroid excretion was 13.4 mg. per 24 hours and fell to 3.8 mg. per 24 hours when cortisone was started. Shortly thereafter this treatment was discontinued. Two days later he suffered an episode of fever, chills, nausea, and vomiting. This was radiologically diagnosed as a "virus pneumonia." At 7$\frac{3}{12}$ years of age the patient had a febrile course with marked weakness. It was also stated that headaches which radiated from frontal to occipital areas took place at least 3 times a month. They were said to occur more often in summer. Aspirin provided slight relief. There was no history of convulsions, paralysis, tics, or visual disorders. On physical examination at 7$\frac{9}{12}$ years of age he was well developed, well nourished, and intelligent but was large and appeared older than his chronologic age (Fig. 3). There was acne around the face and forehead. His muscles were developed very well. Blood pressure was 110/65. The height-age, weight-age, and bone-age ratios were all approximately 14 years at his chronologic age of 7$\frac{9}{12}$ years (Fig. 4). Areolae and nipples were normal. Pubic hair was present in moderate amount. His penis was enlarged to about 10 cm. in length. Scrotum and testes were normal in size for his chronologic age. Hematologic examination revealed a differential count of eosinophils, 6 per cent; neutrophils, 39 per cent; basophils, 1 per cent; lymphocytes, 51 per cent; and monocytes, 3 per cent. The glucose tolerance curve was normal. Serum sodium was 139 mEq. per liter and potassium was 3.3 mEq. per liter. Urinary 17-ketosteroids averaged 25 mg. per 24 hours on 4 successive days. The average value of total plasma 17-ketosteroids was 82 $\mu$g per 100 ml.[15] When cortisone therapy was given both urinary and plasma 17-ketosteroids fell sharply,

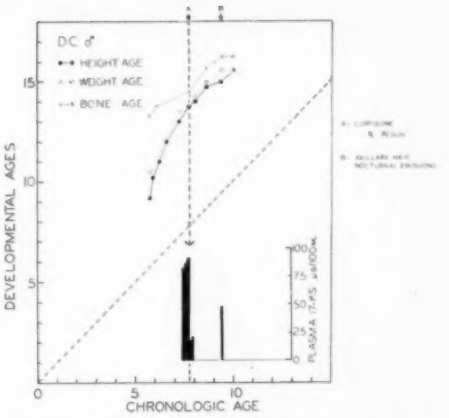



**Fig. 4.** Chart showing growth and developmental progress of D. C., a boy with virilizing adrenal hyperplasia and periodic fever.

as documented in a previous publication.[11]

During this hospitalization period the child was afebrile but complained for 1 day of moderately severe epigastric pain which he described as dull and constant. Physical examination revealed moderate tenderness to deep palpation all over the abdomen. Cortisone treatment was maintained for about 10 months. During this period the child had some spells of weakness and limpness. At age 8$\frac{9}{12}$ years he was again brought to the hospital for re-evaluation. His height was that of a 14-year-old. There was no axillary hair. Pubic hair was abundant; the penis was about 10 cm. long and the testes 1.7 and 1.5 cm. in length. The urinary 17-ketosteroids (4 weeks after discontinuance of cortisone) were 29 mg. per 24 hours. After discharge cortisone was again started at a dose of 20 mg. twice daily by mouth.

At 9 years of age the patient had a paroxysmal episode characterized by burning pain around his face, orbits, eyes, and over the head, with marked photophobia and profuse sweating. Pressure of any nature on the forehead would cause pain. This episode came suddenly, lasted for 2 days, and then suddenly disappeared. The mother had described similar episodes in this boy since infancy, but this was the first attack observed at this institution. A second similar

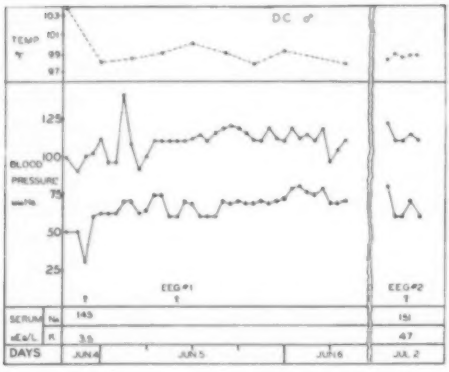



**Fig. 5.** Temperature, blood pressure, and serum sodium and potassium concentrations of D. C. during a typical episode of periodic fever. Note initial hypotension.

episode came 3 months alter and lasted for about a week. There was a delirious state for 3 days and a fever up to 40.5° C. There was no sign of infection on either occasion.

At 9½ years of age the patient was again hospitalized with a similar episode of burning head pain, fever, and chills for the previous 24 hours. On examination the face was flushed and the skin warm. The blood pressure was 100/30, and after 2 hours it was 110/62. The white cell count was 13,500 per cubic millimeter. The fever subsided in 24 hours (Fig. 5).

Six weeks later the child had another episode, with headache followed by fever, shaking chills, restlessness, and abdominal pain. On examination the skin was hot, moist, and well tanned. There was an acneiform eruption over the chest. The blood pressure was normal. The abdomen was flat, with a diffuse tenderness to palpation, guarding, and slight spasm. The white cell count was 15,700 per cubic millimeter; the blood sugar was 100 mg. per cent. The fever lasted for 1 day and the child was discharged 2 days later.

During the following 4 months the patient complained of headaches 2 or 3 times weekly and had notable loss of energy during play and spells of dizziness and momentary loss of vision while at school. At 10 years of age he was again hospitalized for another similar attack which started 3 days previously. There was a low diastolic blood pressure, which in a period of 10 hours rose from a value of 104/30 to a value of 120/82. The leukocytosis, which always had been present on the other admissions, was absent this time. Two months later the child was again admitted with the usual symptoms and signs: flushing of face and conjunctivae, headache, abdominal pain, and nausea. The flushing was so intense that the face appeared edematous. The white blood cell count was 17,850, with 60 per cent neutrophils and 39 per cent lymphocytes. Special studies of plasma 17-ketosteroids will be described later.

Since the child had been on oral hydrocortisone treatment during this period without any relief of the attacks and on the assumption that perhaps there could be a relationship between the administration of this drug and the increased frequency of these episodes, it was decided to stop the administration of hydrocortisone. The following 2 years the patient was free from the episodes, with occasional headache of moderate intensity. This was in marked contrast to the repeated and severe attacks he had had for the few years prior to this time. On physical examination at 12 11/12 years of age he was heavy muscled and short. The blood pressure measured in each arm was 124/64. The genitalia had the appearance of those of an adult male, with profuse pubic and axillary hair. The penis was 10½ cm. long and 3½ cm. wide. Both testes were enlarged and of about equal size. The right testis was 4 cm. by 4 cm. and was of normal consistency. It was very much larger than a plasticene model of the right testis made at the age of 7. Evidence of nocturnal emissions was reported. The urinary 17-ketosteroids were 50 mg. per 24 hours. The bioassay of urinary gonadotropins revealed an excretion of 8 M.U. per 24 hours.

At 14 years of age the patient complained of low back pain for the previous 9 months. No specific x-ray findings were noted. An orthopedic consultant suggested that he discontinue athletic activities and wear a corset.

After a period of 4½ years, during which he had had no corticosteroid therapy and during which he was also free of paroxysmal attacks of periodic fever, he had another episode at 14½ years of age. There was fever, flushing, generalized body aching, shaking chills, and chest pain with dyspnea. His temperature reached 40.5° C. but fell to near normal 5 hours following administration of aspirin and sponging. Within 12 hours he was recovered fully. There was no apparent associated infection. When last seen at the age of 14 10/12 years he stated that joint and back pains had decreased in intensity and frequency. He had resumed occasional participation in sports without ill effects. Headaches were reported as much less frequent. There was some bulbar conjunctival redness bilaterally. Pubic and axillary hair were fully developed. Testes and penis were of adult size and an adolescent beard growth was present. There had been no resumption of corticosteroid therapy.

*Comment.* This boy, in addition to a typical clinical picture of virilizing adrenal hyperplasia in a male, presented periodic episodes of fever, headache and/or abdominal pain, chilly sensation and sometimes shaking chills, flushing of the face, head, ears, and prostration. Occasionally he was nauseated and vomited and on 2 other occasions showed a diastolic hypotension. In most of these attacks there was a leukocytosis. These episodes came suddenly, lasted from 1 or 2 days to a week, and terminated in rapid recovery. A causal relationship of these attacks could not be demonstrated with an infectious state, except in one instance. One episode was related to a blow on the head from a basketball. It was also the impression of some observers that the patient was more prone to have episodes following emotional excitement. Some elements of these attacks resembled so closely an episode of histamine poisoning that a hypothesis was developed interrelating them with the sudden liberation of a histamine-like substance into his plasma.[11]

Kappas and co-workers[16] observed that the intramuscular injection of 100 mg. of etiocholan-3α-ol-17-one (etiocholanolone) produced within the first 6 hours shaking chills and locally heat, swelling, and tenderness; in the next 5 hours there was weakness, muscular aches and pain, sweating headache, and fever up to 39.3° C. These systemic reactions disappeared 18 hours later. There was also increased diuresis and excretion of creatinine and leukocytosis with a shift to the left. Patients who received 20 mg. of etiocholanolone per day for 2 weeks developed a significant eosinophilia. Tolerance for this steroid did not develop, and fever could be maintained as long as its administration was continued. The effects of 25 mg. of etiocholanolone could be significantly diminished by pretreatment with large doses (300 mg. per day) of cortisone for 5 days. The hyperpyrexia was not prevented when this dose of cortisone was given the day of the injection. The local tenderness and the febrile response could be significantly inhibited by the simultaneous administration of aspirin.

Bondy and colleagues[17] have reported 2 adult patients with episodes of periodic fever, malaise, and leukocytosis in whom unconjugated etiocholanolone was demonstrated in plasma during the febrile period. In one of them, the chromatographic fractionation of the urinary 17-ketosteroids during the febrile phase revealed one main peak representing etiocholanolone. When one of the patients was investigated between attacks, etiocholanolone in the unconjugated form was not present in plasma, and there was a normal distribution of urinary 17-ketosteroids. They suggested that the febrile episodes were related to the presence of unconjugated etiocholanolone in plasma.

Our patient has repeatedly presented almost exactly the same symptoms and signs as those elicited by the administration of etiocholanolone. At the time of his first hospitalization his blood smear revealed 6 per cent eosinophils. Determinations of 17-ketosteroids were made separately on the same specimen of plasma by the techniques of Migeon and Plager[18] and of Gardner.[15] The plasma 17-ketosteroid concentration was

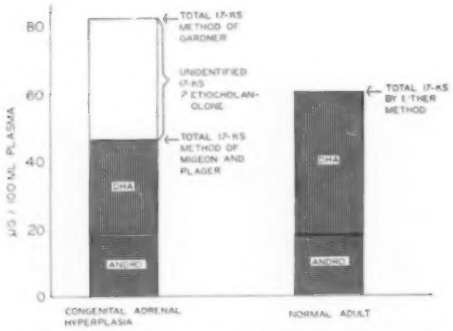



**Fig. 6.** Plasma 17-ketosteroid values obtained with 2 different techniques on a patient with congenital adrenal hyperplasia and periodic fever (D. C.).

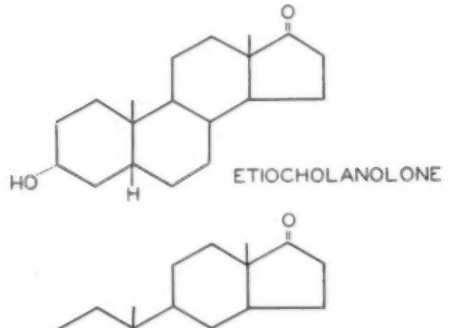



**Fig. 7.** Comparison of structural formulas of etiocholanolone (etiocholan-3α-ol-17-one) and androsterone (androstane-3α-ol-17-one) showing stereoisomerism. The different spatial orientation of the hydrogen attached to carbon[5] is responsible for very different physiologic and pharmacologic effects.

greatly elevated for a child his age (Fig. 6). The graph demonstrates the discrepancy in the results obtained by the 2 methods. The method of Migeon and Plager, which measures specifically androsterone and dehydroepiandrosterone in plasma, yielded average values of 45 μg per 100 ml. for the combined concentration of these two steroids. The method of Gardner, which measures total plasma 17-ketosteroids, yielded average values of 82 μg per 100 ml. When normal young adults were tested, a mean value of 58.5 μg per 100 ml. of plasma for androsterone plus dehydroepiandrosterone was obtained by the method of Migeon and Plager and a mean value of 61.0 μg per 100 ml. for total plasma 17-ketosteroids was obtained by the method of Gardner. The findings of Bondy and others support the hypothesis that the discrepant plasma 17-ketosteroid in our patient was etiocholanolone. Fig. 7 compares etiocholanolone with androsterone and indicates that the only difference is the spatial orientation of the hydrogen on carbon[5].

Our patient, while under treatment with corticosteroids, had his adolescent development at about 9 to 10 years of age, when his bone age was 16 years. There was transient unilateral gynecomastia, growth of the testes to adult size, nocturnal emissions, and growth of axillary hair. Although corticosteroid treatment has been stopped, there was continuing adolescent development, and there have been measurable gonadotropins in his urine.

Boys affected with virilizing adrenal hyperplasia go rapidly into adolescent development once treatment is started. This takes place irrespective of their chronologic age, provided their bone age has reached the level at which normal boys reach puberty. Testicular biopsies on such boys have supported these observations.[19]

Some male patients with virilizing adrenal hyperplasia reach puberty without receiving cortisone treatment.[8, 9] The continued progression of adolescent development in our patient after discontinuance of therapy suggests that this might have been the case with him. To end on a happy note, this patient is now 15 years of age and has the musculature and bony architecture of a mature man. He has become an invaluable member of his high school wrestling team.

## DISCUSSION

The last 2 decades have seen the clinical identification of several different subvariants of the classical picture of virilizing adrenal hyperplasia. The 2 types described in the present paper, together with previously di-

scribed subvariants, are listed in Table I. It is now well known that virilizing adrenal hyperplasia is associated with a metabolic error in the biosynthesis of corticosteroids[20, 21] and is genetically transmitted as a recessive trait.[22] It has also been shown that this enzymatic abnormality is different in at least two varieties of this syndrome and correlates well with the clinical pictures.[23] It is hoped that future studies will elucidate the specific biochemical genetic mechanisms responsible for the production of the various clinical patterns.

**Table I.** Subvariants of virilizing adrenal hyperplasia

1. Virilizing adrenal hyperplasia (classical type)
2. Virilizing adrenal hyperplasia showing excessive sodium loss[1, 2]
3. Virilizing adrenal hyperplasia showing late sodium loss (this report)
4. Virilizing adrenal hyperplasia with hypoglycemia[3, 4]
5. Virilizing adrenal hyperplasia with hypertension[5, 6]
6. Virilizing adrenal hyperplasia with periodic fever[11, 12]
7. Virilizing adrenal hyperplasia showing adolescent sexual development without corticosteroid treatment[8, 9]
8. Postnatal virilizing adrenal hyperplasia[7]

## SUMMARY

Two new clinical subvariants of virilizing adrenal hyperplasia are described, together with a current classification of the recognized clinical patterns. The two new subvariants are virilizing adrenal hyperplasia with (1) "late" sodium loss and (2) periodic fever.

1. In the first instance a girl with virilizing adrenal hyperplasia who had shown no overt signs of sodium loss in the first year of life is described. At the age of 14 months she went into a severe sodium-losing crisis, without having a previous history of such episodes. She has required therapy with desoxycorticosterone since that time.

2. The second patient is a boy with virilizing adrenal hyperplasia who, since the early years of life, had episodes of periodic fever, headaches, and/or abdominal pain, chilly sensation and/or shaking chills, flushing of the face, head, and ears, and prostration. He occasionally had nausea and vomiting and in two instances diastolic hypotension. In most of these attacks leukocytosis was present. Plasma 17-ketosteroid analyses suggest that the episodes of periodic fever and other symptoms and signs may be related to the presence of etiocholanolone in plasma. A classification of the different subvariants of virilizing adrenal hyperplasia is presented.

The authors are indebted to Dr. Joseph Cannon and Mary Imogene Bassett Hospital, Cooperstown, New York, for generous assistance and data concerning P. G. Thanks are also due to the following research fellows who at various times contributed to the care and study of the patients reported: Drs. Pierre Brodeur, Romulo F. Gonzales, Robert E. Greenberg, Esten W. Ray, Mary L. Voorhess, and Sheldon G. Leibow.

## REFERENCES

1. Butler, A. M., Ross, R. A., and Talbot, N. B.: Probable Adrenal Insufficiency in an Infant, J. PEDIAT. 15: 831, 1939.
2. Wilkins, L., Fleischmann, W., and Howard, J. E.: Macrogenitosomia Precox Associated With Hyperplasia of the Androgenic Tissue of Adrenal and Death From Corticoadrenal Insufficiency, Endocrinology 26: 385, 1940.
3. White, F. P., and Sutton, L. E.: Adrenogenital Syndrome With Associated Episodes of Hypoglycemia, J. Clin. Endocrinol. 11: 1395, 1951.
4. Wilkins, L., Crigler, J. F., Jr., Silverman, S. H., Gardner, L. I., and Migeon, C. J.: Further Studies on the Treatment of Congenital Adrenal Hyperplasia With Cortisone. III. The Control of Hypertension With Cortisone, With a Discussion of Variations in the Type of Congenital Adrenal Hyperplasia and Report of a Case With Probable Defect of Carbohydrate-regulating Hormones, J. Clin. Endocrinol. 12: 1015, 1952.
5. Wilkins, L., Lewis, R. A., Klein, R., Gardner, L. I., Crigler, J. F., Jr., Rosemberg, E., and Migeon, C. J.: Treatment of Congenital Adrenal Hyperplasia With Cortisone, J. Clin. Endocrinol. 11: 1, 1951.
6. Shepard, T. H., II, and Clausen, S. W.: Case of Adrenogenital Syndrome With Hypertension Treated With Cortisone, Pediatrics 8: 805, 1951.
7. Wilkins, Lawson: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence, ed. 2, Springfield, Ill., 1957, Charles C Thomas, Publisher.
8. Talbot, N. B., Butler, A. M., and Berman,

R. A.: Adrenal Cortical Hyperplasia With Virilism: Diagnosis, Course and Treatment, J. Clin. Invest. **21:** 559, 1942.
9. Grumbach, M. M., and Wilkins, L.: The Pathogenesis and Treatment of Virilizing Adrenal Hyperplasia, Pediatrics **17:** 418, 1956.
10. Wilkins, L., Crigler, J. F., Jr., Silverman, S. H., Gardner, L. I., and Migeon, C. J.: Further Studies on the Treatment of Congenital Adrenal Hyperplasia With Cortisone. II. The Effects of Cortisone on Sexual and Somatic Development, With an Hypothesis Concerning the Mechanism of Feminization, J. Clin. Endocrinol. **12:** 277, 1952.
11. Gonzales, R. F., and Gardner, L. I.: Congenital Adrenal Hyperplasia With Associated Episodes Resembling Histamine Poisoning, Pediatrics **17:** 524, 1956.
12. Gardner, L. I., and Migeon, C. J.: Unusual Plasma 17-Ketosteroid Pattern in a Boy With Congenital Adrenal Hyperplasia and Periodic Fever, J. Clin. Endocrinol. **19:** 266, 1959.
13. Bongiovanni, A. M., and Eberlein, W. R.: Clinical and Metabolic Variations in the Adrenogenital Syndrome, Pediatrics **16:** 628, 1955.
14. Iversen, T.: Congenital Adrenocortical Hyperplasia With Disturbed Electrolyte Regulation. "Dysadrenocorticism," Pediatrics **16:** 875, 1955.
15. Gardner, L. I.: Plasma Neutral 17-Ketosteroids. I. Technique of Estimation, J. Clin. Endocrinol. **13:** 941, 1953.
16. Kappas, A., Hellman, L., Fukushima, D. K., and Gallagher, T. F.: The Pyrogenic Effect of Etiocholanolone, J. Clin. Endocrinol. **17:** 451, 1957.
17. Bondy, P. K., Cohn, G. L., Herrmann, W., and Crispell, K. R.: The Possible Relationship of Etiocholanolone to Periodic Fever, Yale J. Biol. & Med. **30:** 395, 1958.
18. Migeon, C. J., and Plager, J. E.: A Method for the Fractionation and Measurement of 17-Ketosteroids in Human Plasma, J. Clin. Endocrinol. **15:** 702, 1955.
19. Wilkins, L., and Cara, J.: Further Studies on the Treatment of Congenital Adrenal Hyperplasia With Cortisone. V. Effects of Cortisone Therapy on Testicular Development, J. Clin. Endocrinol. **14:** 287, 1954.
20. Bongiovanni, A. M., Eberlein, W. R., and Cara, J.: Studies on the Metabolism of Adrenal Steroids in the Adrenogenital Syndrome, J. Clin. Endocrinol. **14:** 409, 1954.
21. Bongiovanni, A. M.: In Vitro Hydroxylation of Steroids by Whole Adrenal Homogenates of Beef, Normal Man, and Patients With the Adrenogenital Syndrome, J. Clin. Invest. **37:** 1342, 1958.
22. Childs, B., Grumbach, M. M., and Van Wyk, J. J.: Virilizing Adrenal Hyperplasia; a Genetic and Hormonal Study, J. Clin. Invest. **35:** 221, 1956.
23. Bongiovanni, A. M., and Eberlein, W. R.: Defective Steroidal Biogenesis in Congenital Adrenal Hyperplasia, Pediatrics **21:** 661, 1958.

# Study of the adrenal response to ACTH after prolonged treatment with prednisone alone or in combination with ACTH or testosterone

Jean Bertrand, M.D.,* J. Maitrepierre, M.D., and B. Loras, M.D.

LYON (RHONE), FRANCE

CERTAIN clinical or biologic consequences of the widespread use of corticosteroids in therapy have drawn attention to some poorly known points about the physiopathologic effects of these compounds. As early as 1937 Ingle[19-20] had shown that the administration of large doses of adrenocortical extracts to rats produced atrophy of the adrenal cortex; he thought that this phenomenon was probably the result of a depression of the pituitary corticotropic function. Several clinical incidents occurring suddenly at the time of discontinuing prolonged steroid treatment served to put a more disturbing emphasis on these first notions.[13, 15, 26, 31, 34, 36] Several experimental studies were then conducted for the purpose of investigating its pathogenesis. In hypophysectomized rats which were sustained by injections of corticotropin, adrenal atrophy following prolonged cortisone therapy was no longer observed.[25] In normal animals such steroid treatment was shown to be accompanied by a reduction of the concentration of ACTH in the pituitary body.[12, 21] Finally, an intravenous perfusion of hydrocortisone in an Addisonian patient brought about a rapid reduction of the concentration of ACTH in the plasma.[2] Therefore, a double mechanism seems to play a part in the syndrome resulting from the abrupt discontinuation of corticosteroid treatment: (1) an inhibition of the secretion of pituitary corticotropin; (2) an adrenal atrophy which is secondary to it.

For the clinician two questions remain unanswered: How long after discontinuing prolonged steroid treatment does the decrease in adrenal reserve persist? Can this insufficiency be remedied by combining other therapies with the corticosteroid treatment, i.e., regular injections of long-acting ACTH or androgens? With the aim of contributing to the solution of these problems, we have investigated the variations in the adrenal responses to cotricotropic stimulation after prolonged steroid treatment which was stopped abruptly. This was done by performing the ACTH test at varying time intervals after discontinuation of therapy and by combining regular injections of long-acting ACTH or of testosterone propionate with the prednisone.

## METHODS AND MATERIALS

Thirty-four subjects of both sexes from 20 to 70 years of age were studied. They

*From the laboratoire de Recherches Endocriniennes de la Clinique Médicale Infantile de Lyon (Pr. Bernheim), France.*

**Address, Laboratoire de Recherches Endocriniennes, Clinique Médicale Infantile, Hôpital E. Herriot, Lyon (Rhone), France.*

Table I

| No. | Patient | Sex | Age | Prednisone treatment: Dosage | Prednisone treatment: Duration | Combined treatment | Time between discontinuation and ACTH test (hours) | ACTH test in micrograms per 100 ml. of plasma: 0 hour | 2 hours | 4 hours | 6 hours |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Che | F | 45 | 15 mg. | 1 month | — | 12 | <2 | 3 | 9 | <2 |
| 2 | And | M | | 20 mg. | 20 days | — | 12 | 3.7 | 4.8 | 6.8 | 2.3 |
| 3 | Pet | F | 57 | 15 mg. | 10 months | Intra-articular injections | 12 | 5.6 | 20.5 | 30 | 2 |
| 4 | Bau | M | 63 | 20 mg. | 22 days | — | 12 | 4 | 8 | 14.3 | 12.5 |
| 5 | Lan | M | 26 | 20 mg. | 20 days | — | 12 | <2 | 15.6 | 2 | 16 |
| 6 | Cou | M | 20 | 20 mg. | 20 days | — | 12 | <2 | 11.6 | 4.7 | 2.1 |
| 7 | Mie | M | 34 | 20 mg. | 21 days | Testosterone (50 mg. × 4) | 12 | <2 | 9.4 | 21 | 24.5 |
| 8 | Bau | M | 64 | 20 mg. | 19 days | Testosterone (50 mg. × 4) | 12 | <2 | 9.5 | 12.5 | 14.5 |
| 9 | Tre | M | 37 | 20 mg. | 19 days | Testosterone (50 mg. × 3) | 12 | 6.4 | 16 | 8.4 | 16 |
| 10 | Dou | F | 68 | 20 mg. | 23 days | Testosterone (50 mg. × 4) | 12 | 14.4 | 15.3 | 14.9 | 15.4 |
| 11 | Vec | M | 27 | 20 mg. | 21 days | Testosterone (50 mg. × 4) | 12 | <2 | 11.2 | 17 | 8.1 |
| 12 | Lam | F | 67 | 15 mg. | 3½ months | Testosterone (50 mg. every 7 days) | 12 | 2.6 | 10.8 | 5 | 7.6 |
| 13 | Cou | M | 57 | 20 mg. | 17 days | Long acting ACTH (25 mg. × 3) | 12 | 16 | 20.5 | 42 | 28 |
| 14 | Cra | F | | 20 mg.<br>15 mg. | 16 days<br>26 days | Long acting ACTH (30 mg. × 7) | 12 | 8.8 | 6.7 | 26 | 29.5 |
| 15 | Dec | F | 53 | 20 mg. | 21 days | Long acting ACTH (25 mg. × 4) | 12 | <2 | 17.4 | 25 | 24.5 |
| 16 | Bau | M | 63 | 20 mg. | 20 days | Long acting ACTH (25 mg. × 3) | 12 | 13 | 23.2 | 37.5 | 27 |
| 17 | Gal | M | 37 | 20 mg. | 21 days | Long acting ACTH (25 mg. × 4) | 12 | 9.5 | 23.5 | – | 42.5 |
| 18 | Pic | F | 59 | 20 mg. | 21 days | Long acting ACTH (25 mg. × 3) | 12 | 7.5 | 25 | 33.5 | 50 |
| 19 | Gui | F | 66 | 15-40 mg. | 3 years | Intra-articular injections | 36 | <2 | <2 | <2 | 5.5 |
| 20 | Ric | F | 71 | 20 mg. | 3 months | — | 36 | 12.7 | 34 | 23.5 | 15.5 |
| 21 | Rob | F | 68 | 15 mg. | 22 days | — | 36 | 15.6 | 13.6 | 67 | 17 |
| 22 | Meu | M | 65 | 20 mg. | 21 days | Intra-articular injections | 36 | 6.1 | 48 | 45 | 46 |
| 23 | Mor | F | 22 | 15 mg. | 21 days | — | 36 | 13 | 48 | – | 75 |
| 24 | Lar | F | 60 | 15 mg. | 2 months | — | 36 | 7.8 | 6.6 | 9.4 | – |
| 25 | Del-D | M | 67 | 15 mg. | 7 months | — | 60 | 34 | 36.5 | 43 | 66 |
| 26 | Vil | F | 71 | 20 mg. | 21 days | — | 60 | 23.5 | 57 | 51 | 57 |
| 27 | Tou | F | 30 | 20 mg. | 21 days | Intra-articular injections | 60 | 2.5 | 70 | 4.3 | 3.2 |
| 28 | Pla | M | 38 | 15 mg. | 4 months | — | 60 | 13 | 31.5 | 28.5 | 26 |
| 29 | Gui | F | 68 | 15 mg. | 20 days | — | 60 | 6.4 | 4 | 20 | 18 |
| 30 | Lar | F | 60 | 20 mg. | 21 days | Intra-articular injections | 60 | 3.3 | 6.6 | 6.3 | 8.7 |
| 31 | Gir | F | 63 | 20 mg. | 2½ months | — | 6½ days | 22.5 | 32.5 | 43.5 | 52.5 |
| 32 | Gal | M | 42 | 15 mg. | 21 days | — | 6½ days | 4.25 | 21 | 20 | 25 |
| 33 | Rol | M | 34 | 20 mg. | 21 days | — | 6½ days | <2 | 9.4 | 15.4 | 34 |
| 34 | Gen | F | 60 | 15 mg. | 21 days | — | 6½ days | 5.5 | 12 | 10.6 | 4.5 |
| 35 | Gal | F | 65 | 15 mg. | 21 days | — | 6½ days | 17.7 | 23.5 | 34.1 | 45.7 |
| 36 | Fra | M | 37 | 20 mg. | 18 days | — | 6½ days | 18.8 | 24.6 | – | 29.3 |

were hospitalized for various rheumatic conditions. The doses of prednisone administered ranged from 15 to 40 mg. per day, but when therapy was discontinued the dosage was 15 mg. in all cases. The length of the treatment was variable but was never less than 21 days (Table I).

Adrenocortical stimulation with corticotropin was carried out according to the modalities described by Eik-Nes and colleagues[10]: 6-hour perfusion of 25 mg. of ordinary ACTH with blood sampling every 2 hours; the blood samples were collected with heparin, immediately centrifuged, and the plasma was removed; the quantitative analysis of free plasma 17-hydroxycorticosteroids was made by the technique of Nelson and Samuels.[30]

Thirty-three normal subjects were first tested by this method. The results obtained are as follows:

At 0 hour: average concentration = 14.6 μg per 100 ml. of plasma, varying from 5 to 23.

At 2 hours: average concentration = 31.3 μg per 100 ml. of plasma, varying from 23 to 40.

At 4 hours: average concentration = 39.3 μg per 100 ml. of plasma, varying from 34 to 40.

At 6 hours: average concentration = 44.6 μg per 100 ml. of plasma, varying from 35 to 55.

The adrenal responses to ACTH were tested either 12 hours, 36 hours, 60 hours, or 6½ days after discontinuing the therapy. We also compared the adrenal responses 12 hours after discontinuing the therapy of subjects who had received prednisone only and of other subjects in whom we had combined intramuscular injections, either of long-acting ACTH (25 mg. of zinc-ACTH every 5 to 7 days) or of propionate of testosterone (50 mg. every 5 to 7 days) (Table I).

## RESULTS

**Adrenal responses, 12 hours, 36 hours, 60 hours, and 6½ days after discontinuing treatment (Table I and Fig. 1).** It is apparent that the base-line concentrations of corticosteroids in the plasma rise progressively in proportion to the length of time elapsing between discontinuation of therapy and testing:

After 12 hours: average concentration = 2.2 μg per 100 ml. of plasma, varying from <2 to 5.6.

After 36 hours: average concentration = 9.2 μg per 100 ml. of plasma, varying from <2 to 15.6.

After 60 hours: average concentration = 13.8 μg per 100 ml. of plasma, varying from 2.5 to 34.

After 6½ days: average concentration = 11.5 μg per 100 ml. of plasma, varying from <2 to 20.5.

Consideration of the curves of the adrenal responses to ACTH reveals that at the twelfth hour the reactivity is most often insignificant. The curves at the thirty-sixth and sixtieth hours are very inconsistent, indicating not only variations in the degree of adrenal response from patient to patient but also the irregularity in response of a given individual. Normally the increase in corticosteroids in the plasma is progressive following ACTH stimulation. In these cases, on the contrary, we observed either an absence of response (Cases 19, 24, 29, and 30), an exaggerated response (Cases 22, 23, 25, and 26), or an abrupt increase as early as the second hour of perfusion with progressive reduction afterward of the concentration in the plasma. The last perhaps indicates an exhaustion of secretion (Cases 2, 27, and 28); this insufficiency was twice clinically manifested by serious shock during the testing period (Cases 21 and 24). The decrease in corticosteroids in the plasma demonstrated that in these 2 cases the adrenal gland was incapable of responding to stress by corrective hypersecretion. Finally, the curves at 6½ days appear more regular, although in 2 of 6 cases (Cases 32 and 34) the response is inadequate.

The comparison of these various types of responses with the age, sex, type of treatment, or its duration gave no significant correlation. It may be concluded, there-

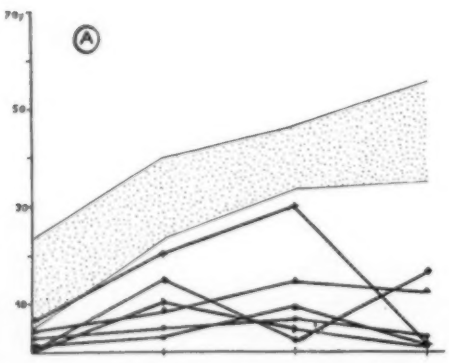



**Fig. 1*A*.** Variations of plasma 17-OHCS during the infusion of 25 mg. of ACTH over a 6-hour period 12 hours after discontinuing prednisone treatment.

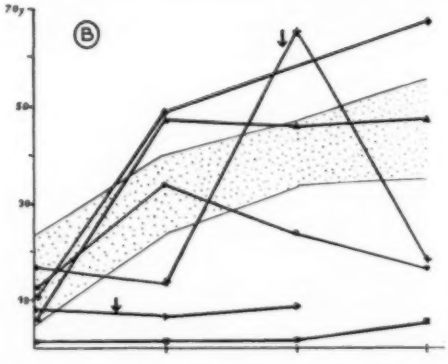



**Fig. 1*B*.** Variations of plasma 17-OHCS during the infusion of 25 mg. of ACTH over a 6-hour period 36 hours after discontinuing prednisone treatment.

fore, that the adrenal gland needs from 3 to 6 days after discontinuation of prolonged steroid treatment to recover its ability to respond normally to ACTH.

**Study of the adrenal responses 12 hours after discontinuing treatment with prednisone combined with long-acting ACTH or testosterone propionate (Table I, Figs. 1A, 2, and 3).** Three series of 6 subjects were compared (Cases 4, 8, and 16 are the same subject); the doses of prednisone were never less than 15 mg. per day and the therapy was always carried out for more than 21 days. In one series regular injections of testosterone propionate (50 mg. every 5 days) were combined with the treatment. In another series 25 mg. of long-acting ACTH were injected every 5 to 7 days during the period of therapy. The third series, previously described, received only prednisone.

It may be seen (Table I) that in the subjects who had received prednisone alone, the average base-line concentration was 2.2 μg per 100 ml. of plasma, varying from less than 2 to 5.6. In the second group, where injections of testosterone propionate were combined with the prednisone, the average concentration was 3.9 μg per 100 ml. of plasma, varying from less than 2 to 14.4, and in the third series, in which the subjects received intramuscular injections of long-acting ACTH in addition to the prednisone, the average concentration was 9.1 μg per 100 ml. of plasma, varying from less than 2 to 16. A simple inspection of the Figs. 1*A*, 2 and 3 readily allows us to establish that the responses are comparable in subjects treated with prednisone alone or in combination with testosterone propionate; they are all clearly inadequate. On the other hand, the simultaneous administration of long-acting ACTH obviously modified the response, since as a whole they can be considered as normal or nearly so.

## DISCUSSION

The duration of the syndrome due to the withdrawal of corticosteroid after prolonged treatment is a problem of the clinician.

Eik-Nes and associates[10] have observed that a normal increase in the concentrations of plasma 17-hydroxycorticosteroids following perfusion of ACTH was obtained 60 hours after prolonged oral cortisone therapy. Christy,[8] Sandberg,[35] Geller,[14] Marks,[28] and respective collaborators have reported somewhat similar results, the general consensus being that about 4 days must elapse after the cessation of steroid treatment in order to obtain a normal response to ACTH infusion. Bierich[3] and Krusius and Oka[22]

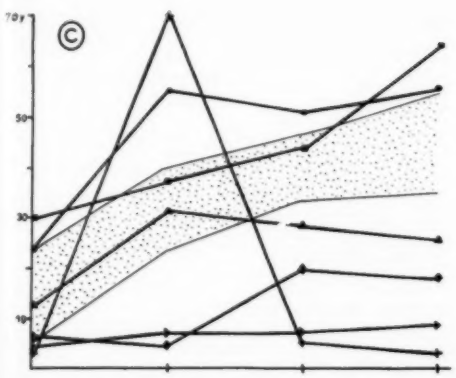



Fig. 1C. Variations of plasma 17-OHCS during the infusion of 25 mg. of ACTH over a 6-hour period 60 hours after discontinuing prednisone treatment.

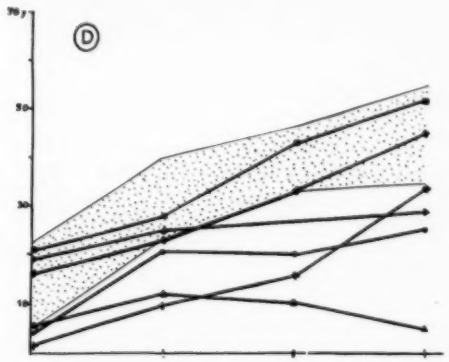



Fig. 1D. Variations of plasma 17-OHCS during the infusion of 25 mg. of ACTH over a 6-hour period 6½ days after discontinuing prednisone treatment. The dotted area shows the limits of variation obtained in 33 normal individuals.

administered the ACTH intramuscularly instead of intravenously. The latter authors believe that 2 to 3 weeks are necessary for a normal response of the adrenal to its physiologic trophic activator. Our study revealed an almost normal response after 6½ days. In addition, our work has shown an extreme individual variability of response to ACTH as well as a marked instability of the concentrations of plasma 17-OHCS during ACTH infusion when serial levels were determined in the same subject; the latter observation occurred particularly at the thirty-sixth and sixtieth hours.

The means of avoiding adrenal atrophy represents the second question which directly interests the clinician; 2 methods were suggested by several experimenters: the use of androgen combined with the cortisone treatment or the simultaneous injection of long-acting ACTH.

Combining testosterone administration with prolonged cortisone treatment in rats prevents the appearance of the adrenal atrophy,[5, 6, 40] but in these conditions the sensitivity to stress is similar to that observed in animals receiving only cortisone.[6] In our study, the results of the ACTH tests in subjects who received either prednisone alone or prednisone combined with 50 mg. of testosterone propionate were practically identical; the administration of testosterone was therefore shown to be unable to prevent adrenal insufficiency which is secondary to prolonged steroid therapy.

The simultaneous administration of ACTH has been studied in experimental animals. Lewis and co-workers[25] observed in hypophysectomized rats that combined therapy with cortisone and ACTH did not result in adrenal atrophy. Similar conclusions were drawn from the work of Hohlweg and Laschet[16] and of Collins[6] in opposition to those of Kracht.[22] Kitay and colleagues[21] have reported that the administration of ACTH to normal rats produced a 95 per cent increase in the concentration of corticotropin in the pituitary gland, whereas administration of cortisone under the same conditions provoked a drop of 60 per cent; simultaneous administration of ACTH and cortisone was also accompanied by a clear diminuition of the pituitary content of ACTH.[18] Exogenous ACTH would appear to inhibit the output of corticotropin, bringing about its accumulation inside the pituitary body; the inhibition of pituitary secretion obtained in this manner would not last more than 24 hours after discontinuing the treatment.[18]

The usefulness of giving injections of long-acting ACTH during prolonged corti-

sone treatment has been pointed out by several authors: Wolfson[38] in 1954, then Young and associates[39] in 1957 advised 160 to 200 mg. of ACTH a week. Bierich[3] observed normal adrenal responses in children when adding 20 units of ACTH twice a week. On the contrary, Marks[28] quoting Hayes and Vermeulen[37] reported no beneficial effect from administration of ACTH. In our experience, combined treatment with long-acting ACTH appeared to be beneficial (Table I).

It must be recalled, however, that the lack of response of the levels of plasma 17-hydroxycorticosteroids to ACTH administration is due to a glandular atrophy which is secondary to the inhibition of pituitary secretion. ACTH tests permit the evaluation of the responsiveness of the adrenal cortex but do not help in the differentiation of adrenal atrophy secondary to pituitary deficiency. We will have to turn therefore to tests examining the whole of the adrenopituitary system. Use of an inhibitor of the 11$\beta$-hydroxylase (SU 4885) now permits estimating indirectly the corticotropic functioning of the pituitary body, provided the adrenal gland is in good functioning order. This produces a drop in adrenal secretion of hydrocortisone which results in stimulation of the anterior pituitary. The adrenal gland is now capable only of liberating 11-desoxy precursor of cortisol (compound S) into the circulation. Its concentration in the urine (assayed as Porter-Silber chromogens) then rises, a reflection of the adrenal stimulation by ACTH. Liddle[27] tested 9 normal subjects this way during or at the end of prolonged treatment with corticosteroids; there was no response to SU 4885 during the therapy, and upon its discontinuance, a refractory period of 1 to 3 days was noted: thus absence of increase in the 17-OHCS in these cases indicates pituitary or adrenal inertia.

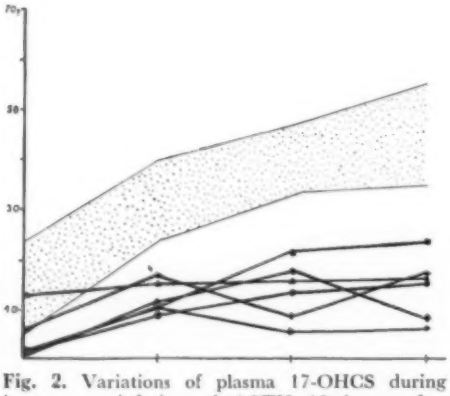


**Fig. 2.** Variations of plasma 17-OHCS during intravenous infusion of ACTH 12 hours after discontinuing combined treatment with prednisone and testosterone propionate (50 mg. every 5 days).

Holub[17] conducted tests with SU 4885 in 4 subjects whose prolonged steroid treatment had been ended by administration of ACTH for 3 days. Three days after the suspension of all therapy, the SU 4885 produced an increase in ketogenic corticosteroids in the urine in 3 cases. The refractory fourth patient had been treated with steroids for 10 years.

Finally, the paradoxical results of the studies of Amatruda and co-workers[1] should be recalled. They observed, even in the immediate period following discontinuation of steroid therapy, a normal increase in the plasma corticosteroids in response to hypoglycemia. On the contrary, only weak corticoadrenal responses to induced hyperthermia were obtained, even when the adrenal gland was stimulated at the end of the cortisone treatment by injections of ACTH.[13]

## SUMMARY

In six series of 6 subjects each who received prolonged treatment with prednisone for various rheumatic conditions their adrenocortical responsiveness was tested at the end of the treatment by an intravenous perfusion of ACTH. Quantitative analyses of plasma 17-hydroxycorticosteroids were made prior to and during the infusion. Twelve hours after discontinuation of therapy there was no response; after 36 and 60 hours the curves were very disordered and 6½ days after discontinuation, the responses, although subnormal, were more regular. Adrenal unresponsiveness cannot be prevented by administration of testosterone

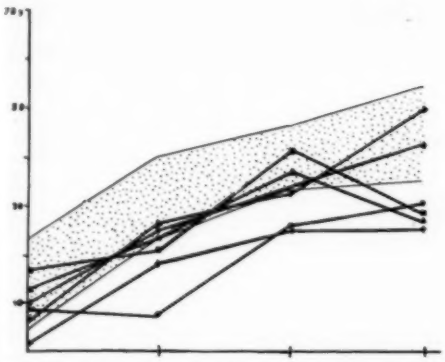


**Fig. 3.** Variations of plasma 17-OHCS during intravenous infusion of ACTH 12 hours after discontinuing combined treatment with prednisone and long-acting ACTH (25 mg. every 5 or 7 days).

concurrently with the corticosteroid therapy; on the contrary, combining regular injections of long-acting ACTH (25 mg. every 5 to 7 days) resulted in normalizing adrenal reactivity to corticotropin 12 hours after discontinuation of the corticosteroid treatment.

## REFERENCES


1. Amatruda, T. T., Hollingsworth, D., D'Esopo, N., Upton, G. V., and Bondy, P. K.: Evidence for Integrity of Hypothalamic Pituitary Adrenal System After Steroid Withdrawal, Clin. Res. **6:** 253, 1958.
2. Bethune, J. E., Nelson, D. H., and Thorn, G. W.: Plasma Adrenocorticotrophic Hormone in Addison's Disease and Its Modification by the Administration of Adrenal Steroids, J. Clin. Invest. **36:** 1701, 1957.
3. Bierich, J. R., Kersten, I., and Maruektad, S.: Plasma Corticosteroids and Their Responsiveness to Corticotrophin After Long-term Therapy With Corticosteroids and Corticotrophin, Acta endocrinol. **31:** 40, 1959.
4. Birke, G., Domeij, B., Olhagen, B., and Plantin, L. O.: Measures for the Avoidance of Adrenal Atrophy in Prolonged Corticosteroid Therapy, Acta med. scandinav. **155:** 245, 1956.
5. Collins, E. J., and Olson, K. J.: Inhibition of Steroid Induced Adrenal Hypofunction, Proc. Soc. Exper. Biol. & Med. **87:** 76, 1954.
6. Collins, E. J.: Steroid Induced Adrenal Pituitary Hypofunction, Endocrinology **58:** 777, 1956.
7. Coste, F., Delbarre, F., and Basset, G.: Recherches sur l'aplasie surrénalienne provoquée par les hormones anti-inflammatoires (cortisone, hydrocortisone, méta-cortandracine), Semaine Hôp. Paris **31:** 2713, 1955.
8. Christy, M. P., Wallace, E. Z., and Jailer, J. W.: Comparative Effect of Prednisone and of Cortisone in Suppressing the Response of the Adrenal Cortex to Exogenous Adrenocorticotropin, J. Clin. Endocrinol. **16:** 1059, 1956.
9. De Filippis, V., and Young, I. I.: Evolution of Adreno-cortical Function With Intramuscular Injection of ACTH Gel, New England J. Med. **257:** 1, 1957.
10. Eik-Nes, K., Sandberg, A. A., Migeon, C. J., Tyler, F. H., and Samuels, L. T.: Changes in Plasma Levels of 17-Hydroxycorticosteroids During the Intravenous Administration of ACTH. II. Response Under Various Clinical Conditions, J. Clin. Endocrinol. **15:** 13, 1955.
11. Engel, E., Demanet, J. C., Brichant, J., and Riondel, A. M.: Emploi d'un pyrogène comme test de la stimulation neuro-hypophyso-surrénalienne, Helvet. med. acta **25:** 552, 1958.
12. Farrell, G. L., and Laqueur, G.: Reduction of Pituitary Content of ACTH by Cortisone, Endocrinology **56:** 471, 1955.
13. Fraser, C. G., Preuss, F. S., and Bigford, W. D.: Adrenal Atrophy and Irreversible Shock Associated With Cortisone Therapy, J. A. M. A. **149:** 1542, 1952.
14. Geller, J., Alvarez, A. S., Gutman, A., De Freitas, A., Gabrilove, J. L., and Soffer, L. J.: Effect of Prednisone on Adrenal Responsiveness to ACTH in Normal Subjects and in Patients With Cushing's Syndrome, J. Clin. Endocrinol. **18:** 409, 1958.
15. Henneman, P. H., Wang, D. M., Irwin, J. W., and Burrage, W. S.: Syndrome Following Abrupt Cessation of Prolonged Cortisone Therapy, J. A. M. A. **158:** 384, 1955.
16. Hohlweg, H., and Laschet, U.: Zur Frage der Cortison- und Corticotropin-wirkung auf das hypophysen nebennierenrinden System, Acta endocrinol. **32:** 437, 1959.
17. Holub, D. A., Jailer, J. W., Kitay, J. I., and Frantz, A. G.: Direct and Indirect Estimation of Pituitary Adrenocorticotropin Reserves in Man Following Adrenal Steroid Therapy, J. Clin. Endocrinol. **19:** 1540, 1959.
18. Holub, D. A., Kitay, J. I., and Jailer, J. W.: Effects of Exogenous Adrenocorticotropic Hormone (ACTH) Upon Pituitary ACTH Concentration After Prolonged Cortisone Treatment and Stress, J. Clin. Invest. **38:** 291, 1959.
19. Ingle, D. J., and Kendall, E. C.: Atrophy of the Adrenal Cortex of the Rat Produced by the Administration of Large Amounts of Cortin, Science **86:** 245, 1937.
20. Ingle, D. J.: Effects of Administration of Large Amounts of Cortin on the Adrenal Cortices of Normal and Hypophysectomized Rats, Am. J. Physiol. **124:** 369, 1938.
21. Kitay, J. I., Holub, D. A., and Jailer, J. W.: Inhibition of Pituitary ACTH Release: An

Extra-adrenal Action of Exogenous ACTH, Endocrinology **64:** 475, 1959.

22. Kracht, J.: Restitution der corticoidbedingten Nebennierenrindenatrophie, Naturwissenschaften **45:** 61, 1958.
23. Krusius, F. E., and Oka, M.: Effect of Long Term Treatment With Corticosteroids on Adreno-cortical Function, Ann. Rheumat. Dis. **17:** 184, 1958.
24. Larzelere, R. G., Barthold, E. A., Willett, F. M., Feichtmeir, T. V., Wilson, L., and Engleman, E. P.: Adrenocortical Function in Long Term Treatment With Corticoids, Arch. Int. Med. **99:** 888, 1957.
25. Lewis, R. A., Rosenberg, E., and Wilkins, L.: The Effects of 17-Hydroxy-11-Desoxycorticosterone Upon the Adrenals of Normal and of Hyophysectomized Rats Maintained With ACTH, Endocrinology **47:** 414, 1950.
26. Lewis, L., Robinson, R. F., Yee, J., Hacker, L. A., and Eisen, G.: Fatal Adrenal Cortical Insufficiency Precipitated by Surgery During Prolonged Continuous Cortisone Treatment, Ann. Int. Med. **39:** 116, 1953.
27. Liddle, G. W., Estep, H. L., Kendall, J. W., Williams, W. C., and Townes, A. W.: Clinical Applications of a New Test of Pituitary Reserve, J. Clin. Endocrinol. **19:** 875, 1959.
28. Marks, L. J., Donovan, M. J., Duncan, F. J., and Karger, R.: Adreno-cortical Response to Surgical Operation in Patients Treated With Corticosteroids or ACTH, Prior to Surgery, J. Clin. Endocrinol. **19:** 1458, 1959.
29. Nelson, D. H., Samuels, L. T., Willardson, D. G., and Tyler, F. H.: The Levels of 17-OHCS in Peripheral Blood of Human Subjects, J. Clin. Endocrinol. **11:** 1029, 1951.
30. Nelson, D. H., and Samuels, L. T.: A Method for the Determination of 17-Hydroxycorticosteroids in Blood, J. Clin. Endocrinol. **12:** 519, 1952.
31. Plumer, J. N., and Armstrong, R. S.: Adrenocortical Failure Following Long Term Steroid Therapy, Arizona Med. **14:** 202, 1957.
32. Richards, J. B., and Pruitt, R. L.: Hydrocortisone Suppression of Stress Induced Adrenal 17-Hydroxycorticosteroid Secretion in Dogs, Endocrinology **60:** 99, 1957.
33. Rinne, U. K., and Näätänen, E. K.: The Effect of Nor-Androstenolone-Phenyl-propionate on the Atrophy of the Adrenal Cortex and Inhibition of Growth Induced by Cortisone Acetate, Acta endocrinol. **27:** 423, 1958.
34. Salassa, R. M., Keating, F. R., and Sprague, R. G.: Clinical Aspects of Suppression of Adrenal Cortical Function After Use of Cortisone, Proc. Staff Meet. Mayo Clin. **28:** 662, 1953.
35. Sandberg, A. A., Eik-Nes, K., Migeon, C. J., and Koepf, G.: Plasma 17-Hydroxycorticosteroids in Hyperfunction, Suppression, and Deficiency of Adrenal Cortical Function, J. Lab. & Clin. Med. **50:** 286, 1957.
36. Slaney, G., and Brooke, B. N.: Postoperative Collapse Due to Adrenal Insufficiency Following Cortisone Therapy, Lancet **1:** 1167, 1957.
37. Vermeulen, A.: Adrenal Hypofunction After Corticoid Withdrawal, Acta endocrinol. **28:** 321, 1958.
38. Wolfson, W. G.: The Prevention of Adrenal Atrophy During Prolonged Treatment With Large Doses of Oral Hydrocortisone, J. Lab. & Clin. Med. **44:** 956, 1954.
39. Young, I. I., De Filippis, V., Meyer, F. L., and Wolfson, W. G.: Maintenance of Adrenal Cortical Responsiveness During Prolonged Corticoid Therapy, Arch. Int. Med. **100:** 1, 1957.
40. Zizine, U.: Action comparative des androgènes, des oestrogènes et de la progesterone sur l'atrophie surrénalienne produite par la cortisone, Compt. rend. Soc. de biol. **146:** 910, 1952.

# *Sexually deviant behavior in Klinefelter's syndrome*

**H. David Mosier, M.D.,* Lawrence W. Scott, B.S., and**

**Harvey F. Dingman, Ph.D.**

LOS ANGELES, CALIF.

THE term, Klinefelter's syndrome, prior to 1955, denoted the clinical complex consisting of idiopathic testicular tubular fibrosis, gynecomastia, and elevated gonadotropin excretion in adult males.[1] Since 1955, it has been known that some of these individuals have positive sex chromatin† and the term has come to be loosely applied to both pre- and postpubertal individuals who are phenotypic males and have positive chromatin.[2]

The chromatin finding in Klinefelter's syndrome stimulated pediatric interest, which has been heightened by several recent observations. Independent chromatin surveys in groups of mentally deficient males have shown a higher proportion of chromatin-positive individuals among the mentally defective than in the general population.[3-7] Table I summarizes the results of these investigations. Very recently direct chromosomal observation has produced evidence that Klinefelter's syndrome has an XXY chromosomal complement.[8] Klinefelter's syndrome has occurred in the same sibship with Mongolism and twinning.[6] Chromosomal studies in Mongolism have shown an abnormal number of somatic chromosomes[9-11] and in one patient in whom both Klinefelter's syndrome and Mongolism were present the chromosome abnormalities characteristic of both conditions were found.[10] Identical twins with Klinefelter's syndrome have been reported.[12]

These observations establish that Klinefelter's syndrome is a genetic disorder and raise the possibility that it is etiologically related to Mongolism and possibly to twinning. The association with mental deficiency is a matter of considerable interest.

Studies of the personality in Klinefelter's syndrome have been few. Limited observations indicate these individuals tend to have a passive aggressive personality.[13, 6] This, however, throws little light on the question whether there is a personality peculiar to Klinefelter's syndrome. Recent review of the cases reported by Mosier and colleagues[6] has shown that a high proportion of their records list sex offenses prior to institutionalization. Further study of the significance of these observations has been done in order to throw some light on the personality pattern in the disorder. This report gives the results of that study.

*From the Department of Pediatrics, University of California School of Medicine, Los Angeles, Calif., and the Pacific State Hospital, Pomona, Calif.*

*Supported by a grant from the National Research Council (S-58-16).*

**Address, Department of Pediatrics, University of California School of Medicine, Los Angeles 24, Calif.*

*†Positive sex chromatin = female sex chromatin.*

## METHODS

**Part I.** The 10 cases of chromatin-positive Klinefelter's syndrome identified in the previously reported survey of Mosier and associates[6] were compared with a control group composed of all males admitted to the Pacific State Hospital during the fiscal years 1948 to 1952. This group has been subjected to other analyses in a report by Kramer and co-workers.[14] Both the chromatin-positive group and the 1948 to 1952 admission group were divided into I.Q. ranges on the basis of admission I.Q. tests. The incidence of sex offenses committed prior to admission was then tabulated for each I.Q. range.[15] Data processing was carried out by one of us (H. E. D.) through the Population Movement Project at the Pacific State Hospital. The difference in incidence of sex offenses between the 2 groups in the I.Q. range of 30 to 69 was evaluated for significance by Fisher's exact treatment of 2 by 2 contingency tables.[16]

**Part II.** Sex chromatin determinations were performed by the oral smear technique on 600 unselected patients classified as Sexual Psychopaths at the Atascadero State Hospital, Atascadero, California. Each patient found to have a positive sex chromatin pattern was examined by one of us (L. W. S.). Urinary 17-ketosteriod and gonadotropin excretion were determined when possible.* Hospital records and attending personnel were consulted for psychiatric data and other patient information.

## RESULTS

**Part I. Comparison between Klinefelter's syndrome and a control group of mental defectives.** Seven of the 10 chromatin-positive patients had a history of sexual offenses prior to admission. This was a higher proportion listing sex offenses than was found in the control population, composed of the total male admissions to the same hospital in the fiscal years 1948 to 1952 (Table II). The patients in the I.Q. range 30 to 69 are shown in a 2 by 2 arrangement in Table III. When these are evaluated by Fisher's exact treatment of 2 by 2 contingency tables, the calculated probability of this arrangement by chance alone is less than 1 in 400 times. This is strong evidence that sex deviancy is more commonly found in institutionalized mentally deficient males with positive sex chromatin (Klinefelter's syndrome) than in the corresponding population of males with negative chromatin.

**Part II. Sex chromatin results in sexual psychopaths.** Six phenotypic males, among the 600 sex psychopaths who were tested, were found to have a positive sex chromatin pattern, an incidence of 1.0 per cent. Table IV gives social and psychiatric information obtained from the records of the positive cases.

The positive cases all had normally developed axillary and pubic hair and normal maturation of the scrotum, penis, and prostate. The body proportions tended to be eunuchoid. The testes were small and firm with the exception of one patient whose testes, though small, had normal consistency. None of the positive group had gynecomastia. The 17-ketosteroids in 3 patients were normal or low normal. Gonadotropin excretion was in the high normal range in the 2 patients tested (Table V).

## DISCUSSION

Independent studies have provided strong evidence that Klinefelter's syndrome is found more frequently among mentally deficient men than in the general population. The

**Table I.** Sex chromatin surveys among mentally deficient men

| *Study* | *No. tested* | *No. of positives* | *% Positive* |
|---|---|---|---|
| Ferguson-Smith[3] | 325 | 4 | 1.2 |
| Prader et al.[4] | 336 | 8 | 2.4 |
| Ferguson-Smith[5] | 663 | 8 | 1.2 |
| Mosier, Scott, and Cotter[6] | 1,252 | 10 | 0.8 |
| Barr[7] | 1,500 | 15 | 1.0 |
| Total | 4,076 | 45 | 1.1 |

*17-ketosteroid gonadotropin and determinations were done by Bio-Science Laboratories, Los Angeles, Calif.

**Table II.** Sex offenses among hospitalized mentally defective chromatin-positive men compared with the general hospitalized population of mentally defective men

| | *Males admitted 1948-1952* | | | *Males tested for sex chromatin* | | | |
|---|---|---|---|---|---|---|---|
| *I.Q.* | *No. admitted* | *No. with sex offenses* | *% of admissions with sex offenses* | *No. tested* | *No. of positives* | *No. of positives with sex offenses* | *% of positives with sex offenses* |
| 0-19 | 117 | 2 | 1.7 | 314 | 0 | 0 | 0 |
| 20-29 | 42 | 3 | 7.1 | 216 | 2 | 1 | 0 |
| 30-49 | 91 | 9 | 9.9 | 396 | 3 | 3 | 100 |
| 50-69 | 124 | 33 | 26.6 | 259 | 5 | 3 | 60 |
| 70+ | 30 | 8 | 26.7 | 37 | 0 | 0 | 0 |
| Unknown | 3 | 0 | 0 | 30 | 0 | 0 | 0 |
| Total | 407 | 55 | 13.5 | 1,252 | 10 | 7 | 70 |

study of Mosier and associates[6] confirmed this. However, it was noted that sexually deviant behavior and not mental deficiency per se accounted for the institutionalization of most of the patients with the syndrome discovered in the survey. It then became important to compare these with other males in the same I.Q. range to see whether a significant difference in the incidence of sexually deviant behavior existed. The results showed that chance alone could not account for the high incidence of sex deviancy in the mentally deficient patients with Klinefelter's syndrome. The question naturally arises whether the personality in Klinefelter's syndrome is particularly prone to lead to sexually deviant behavior. Previous investigation indicated that individuals with Klinefelter's syndrome are likely to have an abnormal personality,[13, 6] yet there has been little evidence that the personality disturbance was anything other than a nonspecific concomitant of the mild mental deficiency often present in the syndrome. The results of this study indicate that the personality disturbance may well be different qualitatively from that of other cases of mental deficiency on the basis of the difference found in respect to sex behavior.

The sex chromatin survey in the group of sex psychopaths unfortunately added little to our knowledge concerning the incidence of sex deviancy in Klinefelter's syndrome in general. The incidence of chromatin-positive Klinefelter's syndrome was essentially that found among mentally deficient males, 1.0 per cent in the sex psychopaths as compared with 1.1 per cent in the combined group of mental defectives reported previously. This is about 5 to 300 times the incidence in the general population depending on the reported estimate for the latter.[3, 4, 17] This finding in the institution for sex psychopaths may not be significant inasmuch as the institutional population may not be representative of the noninstitutionalized sexually deviant population. In dealing with sex offenders, courts may unconsciously select a higher proportion of Klinefelter's syndrome for institutionalization. These men are infertile and are less likely to be shown leniency than are fathers who are responsible for their children; they are also likely to have an abnormal personality pattern and are therefore more readily committed by the court than individuals with apparently well-balanced personalities.

Further work is needed to show the true incidence of sex psychopathy among all patients with Klinefelter's syndrome. Sex chromatin surveys on other institutional groups

**Table III.** Patients in I.Q. range of 30 to 69

| | *Chromatin positive* | *Admission 1948 to 1952* | *Total* |
|---|---|---|---|
| No sex offense | 2 | 173 | 175 |
| Sex offense | 6 | 42 | 48 |
| Total | 8 | 215 | 223 |

might cast additional light on the relationship of behavioral and personality disorders to the syndrome. These groups should include sex offenders imprisoned for other criminal acts, criminally insane, and other psychotic groups. Estimates of the incidence of chromatin-positive Klinefelter's syndrome in the general population should be placed on firmer footing by more adequate surveys of "normal" males. The tedious nature of such a broad survey has undoubtedly discouraged its accomplishment.

Klinefelter's syndrome may be diagnosed in the prepubertal years by sex chromatin testing. Heretofore there has been little basis for performing chromatin testing on immature boys because the pathologic anatomy of Klinefelter's syndrome does not develop until after puberty. The recent discoveries that mental deficiency and serious personality and behavioral disorders frequently accompany the syndrome suggest that boys with these symptoms should have chromatin testing included in their diagnostic studies. Additional emphasis should be given to this when Mongolism or twinning have occurred in the same sibship.

The early recognition of Klinefelter's syndrome will permit observation of the development of the personality pattern and also make it possible to practice preventive psychiatry in these cases. The data obtained

**Table IV.** Social and psychiatric data in cases with positive sex chromatin

| *Patient* | *Race* | *Schooling (grade)* | *Intelligence* | *Psychiatric diagnosis* | *Offense* |
|---|---|---|---|---|---|
| A | Caucasian | Tenth | I.Q.: 83* | Personality pattern disturbance: schizoid personality | Attempted rape of maried woman |
| B | Caucasian | Eighth | I.Q.: 101† | Personality pattern disturbance: schizoid personality | 3 attempted rapes; 2 on his mother |
| C | Caucasian | First college | Normal‡ | Personality trait disturbance: passive aggressive personality | Pedophilia involving boys |
| D | Negro | Eleventh | I.Q.: 95† | Personality trait disturbance: emotionally unstable personality | Sodomy with boys |
| E | Caucasian | Seventh | Dull normal‡ | Sociopathic personality disturbance: sexual deviation | Pedophilia involving girls |
| F | Caucasian | Twelfth | Normal‡ | Sociopathic personality disturbance: sexual deviation | Pedophilia involving boys |

*Oral I.Q. examination.

†Wechsler-Bellevue.

‡Estimated by psychiatric interviewers.

**Table V.** Clinical and laboratory data in cases with positive sex chromatin

| *Patient* | *Age (years)* | *Height (inches)* | *Span (inches)* | *Testis length (cm.)* | *17-ketosteroids (mg./24 hr.)* | *Gonadotropin (m.u./24 hr.)* | |
|---|---|---|---|---|---|---|---|
| A | 24 | 67.3 | 68.0 | 1.0 | 14.7 | > 16 | < 50 |
| B | 30 | 78.5 | 79.0 | 1.0 | 11.6 | > 16 | < 50 |
| C | 32 | 72.0 | 71.0 | 1.0 | 8.5 | -- | -- |
| D | 34 | 67.5 | 73.0 | 2.0 | -- | -- | -- |
| E | 37 | 70.5 | 74.0 | 2.5 | -- | -- | -- |
| F | 45 | 69.0 | 66.0 | 1.0 | -- | -- | -- |

through this experience may assist in understanding the role of genetic factors in personality development.

## SUMMARY

A comparison between 10 mentally deficient individuals with chromatin-positive Klinefelter's syndrome and a control group of mentally defective males showed a much higher incidence of sexually deviant behavior among those with Klinefelter's syndrome. This difference was statistically significant, with a probability of occurrence by chance of less than 0.0025.

A sex chromatin survey on 600 institutionalized male sexual psychopaths showed that 6 or 1.0 per cent were chromatin positive. This incidence, while above that of the general population, may have been brought about by certain social factors tending to favor the commitment of men with the abnormalities found in Klinefelter's syndrome.

Recent observations in Klinefelter's syndrome indicate that chromatin testing should be done on boys with mental deficiency or with serious personality or behavioral disturbances, especially in those instances when Mongolism or twinning has occurred in the same sibship.

The authors express their appreciation to Dr. Reginald S. Rood, Superintendent and Medical Director, Atascadero State Hospital, Atascadero, California, his staff, and the patients for their cooperation in making this study possible.

## REFERENCES

1. Klinefelter, H. F., Jr., Reifenstein, E. C., Jr., and Albright, F.: Syndrome Characterized by Gynecomastia, Aspermatogenesis, Without Aleydigism, and Increased Excretion of Follicle-Stimulating Hormone, J. Clin. Endocrinol. **2:** 615, 1942.
2. Nelson, W. O.: Evaluation of Testicular Function in Reproduction and Sterility, Michigan State University, Centennial Symposium Report, East Lansing, Michigan, 1955, pp. 79-85.
3. Ferguson-Smith, M. A.: Chromatin-Positive Klinefelter's Syndrome (Primary Microrchidism) in a Mental Deficiency Hospital, Lancet **1:** 928, 1958.
4. Prader, A., Schneider, J., Züblin, W., Francés, J. M., and Rüedi, K.: Die Häusigkeit des echten, chromatin-positiven Klinefeltersyndroms und seine Bezeihungen zum Schwachsinn, Schweiz. med. Wchnschr. **88:** 917, 1958.
5. Ferguson-Smith, M. A.: The Prepubertal Testicular Lesion in Chromatin-Positive Klinefelter's Syndrome (Primary Microrchidism) as Seen in Mentally Handicapped Children, Lancet **1:** 219, 1959.
6. Mosier, H. D., Scott, L. W., and Cotter, L. H.: The Frequency of the Positive Sex-Chromatin Pattern in Males with Mental Deficiency, Pediatrics **25:** 291, 1960.
7. Barr, M. L.: Tests of Chromosomal Sex in Klinefelter's Syndrome and in Mental Deficiency, Proc. IX Internat. Cong. of Pediatrics, Montreal, Canada, July 19, 1959.
8. Jacobs, P. A., and Strong, J. A.: A Case of Human Intersexuality Having a Possible XXY Sex-Determining Mechanism, Nature, London **189:** 302, 1959.
9. Lejeune, L., Gautier, M., and Turpin, R.: Le chromosomes humains en culture de tissus, Compt. rend. Acad. Sc. **248:** 602, 1959.
10. Ford, C. E., Jones, K. W., Miller, O. J., Mittwock, U., Penrose, L. S., Ridler, M., and Shapiro, A.: The Chromosomes in a Patient Showing Both Mongolism and the Klinefelter's Syndrome, Lancet **1:** 709, 1959.
11. Jacobs, P. A., Baikie, A. G., Brown, E. M. C., and Strong, J. A.: The Somatic Chromosomes in Mongolism, Lancet **1:** 710, 1959.
12. Holub, D. A., Grumbach, M. M., and Jailer, J. W.: Seminiferous Tubule Dysgenesis (Klinefelter's Syndrome) in Identical Twins, J. Clin. Endocrinol. **18:** 1,359, 1958.
13. Pasqualini, R. A., Vidal, G., and Bur, G. E.: Psychopathology of Klinefelter's Syndrome, Lancet **2:** 164, 1957.
14. Kramer, M., Person, P. H., Jr., Tarjan, G., Morgan, R., and Wright, S. W.: A Method for Determination of Probabilities of Stay, Release and Death, for Patients Admitted to a Hospital for the Mentally Deficient: The Experience of Pacific State Hospital During the Period 1948-1952, Am. J. Ment. Deficiency **62:** 481, 1957.
15. Dingman, H. F.: Unpublished data.
16. Seigel, S.: Nonparametric Statistics for Behavior Sciences, New York, 1956, McGraw-Hill Book Company, Inc.
17. Moore, K. L.: Sex Reversal in Newborn Babies, Lancet **1:** 217, 1959.

# *Ovarian tumors and cysts associated with sexual precocity*

## *Report of 3 cases and review of the literature*

**Walter R. Eberlein, M.D.,* Alfred M. Bongiovanni, M.D., Iris T. Jones, M.D., and William C. Yakovac, M.D.**

PHILADELPHIA, PA.

ISOSEXUAL precocity in girls may be arbitrarily defined as the appearance of feminine secondary sexual characteristics before the age of 7 years or menstrual bleeding before the age of eight. These physical changes may represent the beginning of normal adolescent development at an abnormally early age, with the attainment in childhood years of the ability to reproduce (precocious puberty); or they may reflect instead an autonomous activity of the ovary, rarely the adrenal, or the ingestion of estrogens (pseudoprecocity). Both forms of sexual precocity are mimicked by two normal variants of adolescent development, an idiopathic enlargement of the breasts not associated with other feminine changes (precocious thelarche)[1-3] or the unexplained appearance of sexual hair without other virilization (precocious pubarche or adrenarche).[1, 2]

*From the Children's Hospital of Philadelphia, Department of Pediatrics, School of Medicine, University of Pennsylvania.*

*Supported in part by Grants A-1973 and A-619 (C4) of the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service.*

**Address, Children's Hospital of Philadelphia, 1740 Bainbridge St., Philadelphia 46, Pa.*

Between September, 1954, and July, 1959, 86 girls were referred to the Endocrine Clinic of the Children's Hospital of Philadelphia with one or more signs of early sexual development (Table I). During the same period only 3 boys were referred because of suspected precocity. These figures, which agree with others in the literature,[1, 4-7] indicate how much more common sexual precocity is among girls than boys. Kussmaul[8] first pointed this out in his classic and rarely cited paper. Although one of the earliest to report a case of sexual precocity produced by an ovarian tumor, he emphasized that in most girls no cause was ever found; in a few, the condition was due to "certain affections of the brain." Our experience attests to the accuracy of this old observation.

Table I is intended primarily to serve as a frame of reference within which to discuss three rather uncommon examples of sexual precocity associated with ovarian disease. These cases seem worth reporting, in part because of their rarity, but more especially because they illustrate the difficulties sometimes encountered in trying to distinguish true precocity from pseudoprecocity in the

female, both by physical examination and by the employment of available endocrine tests.

## CASE REPORTS

**Case 1.** Granulosa cell tumor of the ovary. B. P., No. 55822, a white female infant was delivered spontaneously following a normal pregnancy. The birth weight was 8 pounds, and the length, 22 inches. Her early growth and development and the family history were unremarkable. At 2½ years of age she developed a brownish vaginal discharge. Because this recurred over a period of several weeks she was examined under anesthesia by a gynecologist who found no pelvic abnormalities. At age 3 years breast enlargement was first noted, along with increased height gain. A rectal examination as part of a general physical examination at that time was said to be negative. Subsequently she complained of recurrent, vague lower abdominal pain and began to have a persistent white vaginal discharge. Four days prior to admission to this hospital a physician discovered a tender lower abdominal mass.

She was referred to Children's Hospital at age 4 4/12 years. Her height was 47 inches (height age 6 years) and weight, 47.5 pounds. She appeared tall, well nourished, and neither acutely nor chronically ill. Both breasts were enlarged for her age. The left breast was 7.5 cm. in diameter at the base with 3 cm. of glandular tissue beneath the areola, which measured 2 cm. Areolar tubercles and slight pigmentation were present. The right breast measured 4 cm. in diameter at the base, with a small amount of glandular tissue palpable beneath the areola. There was no axillary hair or acne. A small amount of blonde fuzz was noted over the labia but no true sexual hair. The labia majora appeared somewhat fleshly, the labia minora were overdeveloped for her age, and the vaginal mucosa showed marked estrogenization by smear. A firm midline abdominal mass the size of an orange was palpated bimanually. It could be displaced to the right, causing pain.

A routine urinalysis and blood count were normal. Skull x-ray was not remarkable. A flat film of the abdomen revealed an indefinite soft tissue mass in the pelvis but it was not diagnostic. Bone age survey disclosed 9 year centers at the wrist. A 24-hour urine collection contained 13 mcg. estrogens (total fluorogens) and 0.3 mg. 17-ketosteroids; both values are normal for age in this laboratory.

At laparotomy the uterus was found to be enlarged, approximately 2 to 3 times the normal size for age. The right ovary appeared normal grossly. A well-encapsulated tumor replaced the left ovary and was re-

**Table I.** Cases of sexual precocity in girls at Children's Hospital, 1954-1959

| *Initial diagnosis* | *No. of cases* | *Final diagnosis* | *No. of cases* |
|---|---|---|---|
| *I. True precocity* | | | |
| a. Idiopathic | 43 | Idiopathic | 43 |
| b. McCune-Albright syndrome | 4 | McCune-Albright syndrome | 4 |
| *II. Pseudoprecocity* | | | |
| a. Ovarian tumor | 3 | Granulosa cell tumor | 2 |
| | | Theca-lutein cyst | 1 |
| *III. Variants of normal adolescence* | | | |
| a. Precocious thelarche | 25 | Idiopathic precocity | 2 |
| | | Early adolescence | 3* |
| | | Precocious thelarche | 20† |
| b. Precocious pubarche | 11 | Idiopathic precocity | 4 |
| | | Early adolescence | 2* |
| | | Precocious pubarche | 5† |

*"Early adolescence" is defined as isolated appearance of breast enlargement or of pubic hair before 7 years of age, followed by adolescent development between ages 8 and 10.

†Insufficient follow-up of some patients.

**Fig. 1.** Case 1. Granulosa cell tumor.

moved (Fig. 1). The tumor measured 10 by 7 by 5 cm. and weighed 250 grams. The left Fallopian tube was adherent to the capsule. The regional lymph nodes did not appear abnormal. The cut surface of the tumor showed moderately soft, friable, yellowish-white and gray mottled tissue which was hemorrhagic. There were numerous small cysts filled with chocolate-colored fluid. Microscopically (Fig. 2) the appearance of the tumor cells varied in different sections. In many areas the cells were uniform in size with large nuclei; they were relatively deficient in chromatin, but had an adequate amount of pale cytoplasm. The cells tended to be arranged in fasciculi around a lumen containing acidophilic fluid. In other areas the cells were hyperchromatic, of giant size, and bizzare appearing. These sections were reviewed by Professor Arthur T. Hertig, who suggested that this could be an unusual type of granulosa cell tumor or an ovarian carcinoma of the mesometanephric group. He commented on the lack of thecal stroma, considered to be the source of estrogen synthesis in both the normal follicle and in granulosa cell tumors.

On the second and third postoperative days slight vaginal bleeding occurred. The patient was discharged on the fifth postoperative day. She was followed as an out-patient thereafter. When she was seen at 5 3/12 years, she was asymptomatic, her height was 47.5 inches (decreased rate of growth), and weight, 52 pounds. Both breasts had decreased in size and no glandular tissue was palpable. The areolae were no longer pigmented. The labia majora were not remarkable but the labia minora remained prominent. Rectal examination was negative. A 24-hour urine collection was assayed by the rat ovarian-weight method of Albert[9] and found to contain less than 10 gonadotropin units. She was seen again at 6 6/12 years of age. Two months and one month, respectively, prior to this visit she had a slight brownish vaginal discharge lasting one day. Her height was now 50.5 inches and weight, 55 pounds. The breasts were slightly prominent for her age but no breast tissue could be palpated. The external genitals remained unchanged. There was no vaginal discharge. No pubic or axillary hair had appeared. A rectal examination was again negative. It was concluded that the signs of sexual precocity had regressed, only the labia minora remaining somewhat prominent for age.

The child was re-examined at 8 2/12 years of age: during the previous 6 months she had had occasional slight pinkish-brown vaginal discharge for 1 to 2 days, but no frank bleeding. Her height was 53.6 inches and weight, 62 pounds. Breast development had recurred, both breasts measuring approximately 6 cm. at the base with 0.5 cm. glandular tissue. The external genitals remained as before. There was no axillary or pubic hair. On rectal examination there appeared to be slight development of the uterus, the cervix measuring approximately

2 cm. in length. The bone age was 10 to 11 years. A 24-hour urine collection contained 1.3 mg. of 17-ketosteroids; 12.7 mcg. of estriol[10] (adolescent range), and less than 10 gonadotropin units. The impression at this visit was that she was now showing early adolescent changes commensurate with her bone age. There was no evidence of recurrence of the tumor.

**Case 2.** Granulosa cell tumor of ovary. L. A. W. No. 585798, a white female infant born Dec. 18, 1955, weighed 8 pounds and was 20 inches in length. Her early growth and development were normal and the family history was not remarkable. She was admitted to Children's Hospital at 2 8/12 years of age with the complaints of increased breast size and prominence of the abdomen of 2 weeks' duration. The child had occasionally complained of vague lower abdominal pain during the 2 to 3 weeks prior to admission. The mother noted reddening and swelling of the external genitals for 3 days. No vaginal bleeding had occurred.

On admission her height was 36.25 inches (normal for age), weight 36 pounds (average for height, 29 pounds). Striking on physical examination was the marked prominence of the breasts and abdomen. The breasts were equal in size, measuring 5 cm. at the base, with glandular tissue approximately 3 cm. in diameter. Both areolae were slightly enlarged and pigmented. There was no sexual-type labial or axillary hair and no acne. The labia majora were reddened and fleshy appearing, the labia minora prominent. The abdomen measured 24 inches in circumference, compared to the average for her height of 19 inches. An ill-defined mass was felt in the left central and lower quadrants of the abdomen, ballottable from the right to the left. On rectal examination neither the uterus nor the ovaries could definitely be felt.

Blood counts and routine urinalysis on admission were normal. Skull films were negative. X-rays of multiple epiphyseal centers revealed a slightly advanced bone age of 4 years. A vaginal smear showed a moderate estrogenic effect. Several 24-hour urine collections were assayed: 17-ketosteroids 1.6 to 3.1 mg.; estriol 16.0 to 20.3 mcg. (adult

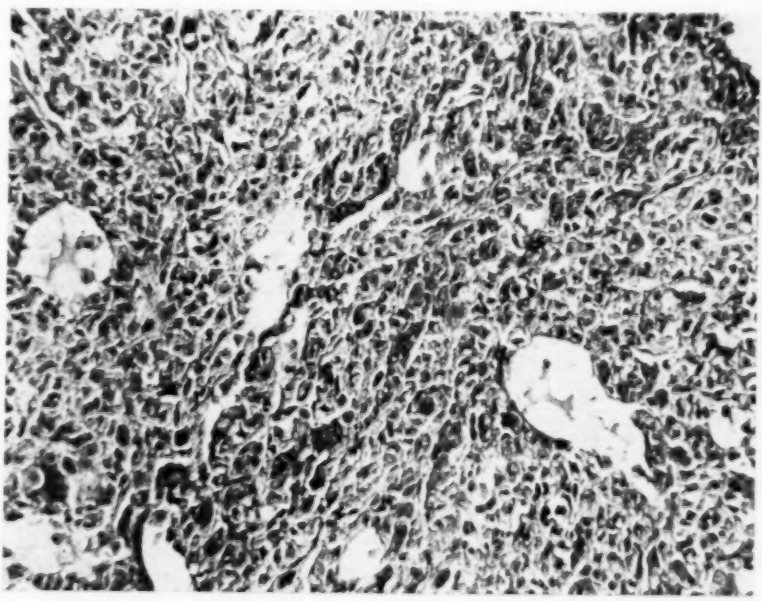


**Fig. 2.** Case 1. Variable appearance of tumor cells, absence of theca cells. (×100; reduced ¼.)

early luteal phase level); pregnanediol[11] 0.14 to 0.34 mg.; and pregnanetriol[12] 0.33 mg. (both normal for age). By bioassay the urine contained 12 gonadotropin units per 24 hours, a normal adult level.

Because of a rapid gain in weight of 4 pounds and an increase of 2 inches in abdominal girth since admission, the child was submitted to exploratory laparotomy on the fourth hospital day. Approximately 4 L. of clear fluid was found in the abdominal cavity. A well-encapsulated tumor of the left ovary, weighing 420 grams, was removed along with the adherent adult-sized Fallopian tube. There was no evidence of regional lymph node metastasis. The right ovary appeared infantile. The uterus was enlarged for age. Postoperatively the child's weight fell to 30 pounds and scant vaginal bleeding occurred on the third to the fifth postoperative days. She was discharged on the sixth postoperative day, at which time urinary estriol excretion was 12.7 mcg. per 24 hours.

On cut surface the yellow, fatty but firm tumor showed areas of necrosis and petechial hemorrhages (Fig. 3). The pale blue capsule appeared thickened and was intact. Microscopic sections (Fig. 4) demonstrated that the tumor consisted of luteinized granulosa cells arranged in trabecular and alveolar patterns. There were occasional mitotic figures and random focal areas of necrosis. The follicles contained lipidlike material and Call-Exner bodies. The sections were reviewed by Professor Hertig who agreed that this was a partially luteinized granulosa cell tumor. As in Case 1 he commented on the paucity of thecal stroma.

The child was seen on follow-up examination at 2 10/12 years of age. She had remained asymptomatic since operation. Her height was 36.25 inches and weight, 30 pounds, both unchanged. The abdominal circumference was 21 inches. The breasts remained unchanged. Only downy hair was noted over the pubis. The vaginal mucosa was pink and moist, the labia minora still prominent, but the labia majora less reddened. On rectal examination the cervix still felt slightly enlarged. The adnexa were unremarkable. A 24-hour urine collection contained 1.6 mg. 17-ketosteroids and 8.3 mcg. estriol.

The patient was seen again at 3 4/12 years of age. She had remained asymptomatic. Her height was 37.5 inches. No breast

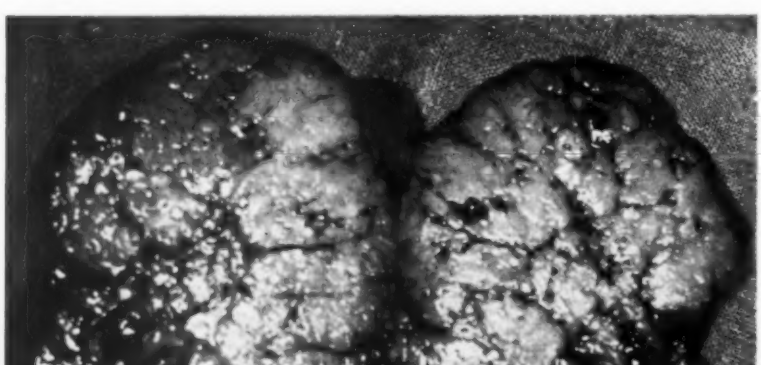


**Fig. 3.** Case 2. Granulosa cell tumor.

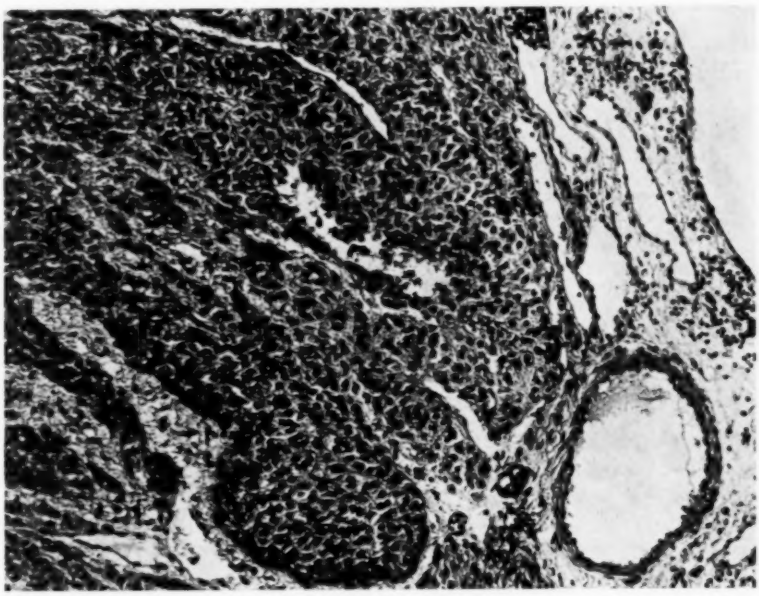


**Fig. 4.** Case 2. Trabecular and alveolar arrangement of tumor cells. (×100; reduced ¼.)

tissue was palpable at this time and the downy pubic hair had disappeared. The labia minora still appeared slightly large for her age, but were unstimulated and the vaginal mucosa was infantile. Rectal examination was negative. Urine assays were 17-ketosteroids 1.3 mg. and estriol 6.2 mcg. per 24 hours (normal range for maturational age). It was felt at the time of this visit that the signs of sexual precocity had almost completely regressed.

**Case 3.** Theca-lutein cyst of ovary. D. K. No. 585795, a white girl born Dec. 15, 1956, had a birth weight of 7 pounds 7 ounces. and a length of 19.5 inches. Her early growth and development were normal. At the age of 9 months she was 29 inches tall, at the age of 1 year she weighed 23.5 lbs. She was in good health until 1 3/12 years of age, when she began to pass dark red-brown liquid blood and clots per vaginam. Bleeding persisted for 8 days until admission to another hospital. There it was noted that the breasts appeared prominent and hair was present over the pubis. A cystoscopic examination under anesthesia was negative. X-rays of the skull and long bones were said to be normal. 17-ketosteroid excretion was reported as 2.0 mg. per 24 hours. Following hospitalization no further bleeding, noticeable growth spurt, or development of pubic hair occurred. The breasts seemed to vary in size from month to month. At 1 8/12 years of age vaginal bleeding recurred, lasting for 5 days, up to the time of admission to this hospital.

On admission her height was 35.75 inches (height age 2 8/12 years) and weight, 28.8 pounds (normal for height). Chest circumference over the nipples was 20.0 inches. Both breasts were prominent, measuring 4 cm. in diameter at the base; the nipples protruded and a small amount of granular tissue could be felt beneath the slightly pigmented areolae, which measured 1.5 cm. in diameter. The labia majora appeared normal but the labia minora were fleshy, and the clitoris was somewhat developed for her age. Downy

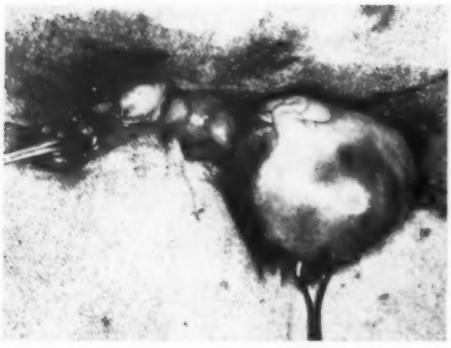


Fig. 5. Case 3. Theca-lutein cyst of left ovary.

pubic and labial hair was present but there was no true sexual hair. There was no axillary hair or acne. The vaginal orifice was partially occluded by a hymen draped with strands of blood-tinged mucus. Nothing abnormal was noted on palpation of the abdomen. On rectal examination the cervix and uterus appeared to be of adolescent size. A large, nontender boggy mass was palpated in the left adnexal area and was poorly delimited from the uterus.

Routine blood counts and urinalysis were normal; chest and skull x-rays were also normal. X-rays of various epiphyseal centers revealed an average bone age of 4 years. A lateral film of the abdomen showed displacement of the sigmoid colon anteriorly by a soft tissue mass arising from the pelvis. A vaginal smear showed moderate estrogen effect. A 24-hour urine collection was found to contain 1.4 mg. 17-ketosteroids and 9.2 mcg. estriol, the latter an adolescent or early follicular phase level. No urinary gonadotropins could be detected by the rat ovarian weight methods.

Laparotomy was performed on the fourth hospital day. The right ovary appeared normal but was biopsied. The uterus was enlarged for her age. The left ovary, aside from a thin layer of tissue at the upper pole, was replaced by a pale blue cyst which measured 5 by 5 by 3 cm., and weighed 41 grams (Fig. 5). The cyst contained old and fresh blood. Biopsy of the right ovary revealed only undeveloped, primordial follicles scattered in a normal stroma, beneath a thin capsule. The cortical remnant of the left ovary was found to possess a thickened tunica albuginea, closely resembling the dense capsule seen in the Stein-Leventhal syndrome, along with immature primary follicles. Sections of the cyst (Fig. 6) demonstrated that it arose from the hilum of the ovary, surrounded by ordinary fibrous tissue extending to the serosa. The cyst wall consisted of several layers of partially luteinized theca interna cells overlying a poorly delimited layer of admixed stromal and granulosa cells. The Fallopian tube removed with the left ovary was lined by tall columnar epithelium, indicative of estrogen stimulation. The sections were reviewed by Professor Hertig who commented on the unusual capsular fibrosis, the lack of any follicular development within the ovarian cortex, and the hilar origin of the cyst.

Scant withdrawal bleeding occurred on the second to the fourth postoperative days. The child was discharged on the sixth day, at which time urinary estriol excretion had fallen to 5.3 mcg. per 24 hours. She was seen in follow-up examination 6 months later, at the age of 2 2/12 years. She had remained asymptomatic since discharge from the hospital. Her height was 37.5 inches (height age 3 3/12 years); weight, 28 pounds, and chest circumference, 19.5 inches. The mother had noted no change in breast size. Both breasts on examination appeared somewhat prominent but only a few strands of granular tissue could be palpated beneath the areolae. The labia minora were the same size but less edematous. The cervix was felt by rectal examination to be slightly enlarged and the adnexa were unremarkable. The bone age was unchanged. A 24-hour urinary assay at this time showed 0.5 mg. of 17-ketosteroids, and 7.4 mcg. of estriol, the latter being slightly elevated for age. It was felt that the sexual precocity had regressed rather than progressed since operation.

## DISCUSSION

The 2 cases of granulosa cell tumor described illustrate a rather rare cause of sex-

ual precocity. The exact incidence of this condition is unknown. Kussmaul[8] in 1862 reported a case of feminizing ovarian "sarcoma" and referred to two other possible cases in the older literature. Prior to the time of von Kahlden (1895) granulosa cell tumors were considered to be either sarcomas or carcinomas. Lenz[4] in 1913, recorded the same 3 cases in Kussmaul's paper. In 1946 and 1949 Seckel[6, 13] mentioned briefly 31 definite cases of granulosa cell tumors producing sexual precocity and 30 more probable instances in the literature. By 1955[14] he had discovered 40 proved cases in addition to 4 old and 1 new case of thecoma. Pedowitz[15] in 1955, including the uncertain but probable cases mentioned by Seckel, referred to 62 granulosa cell tumors, 3 thecomas, 12 ovarian teratomas, and 3 dysgerminomas associated with sexual precocity. He reported an additional case of thecoma, as well as a granulosa cell tumor in a 10-year-old girl.

Since the articles of Pedowitz and Seckel, at least 16 additional case reports of granulosa cell tumor producing sexual precocity have appeared in the American and foreign literature,[14, 16, 18-30] bringing the total to approximately 60 proved and 30 probable cases. If thecomas are histologic variants of granulosa cell tumors, which many pathologists,[31-34] although not all,[35] believe, and if certain ovarian carcinomas, sarcomas, and dysgerminomas represent less well-differentiated forms of the ovarian folliculoma or mesenchymoma,[36] the number of cases of feminizing granulosa cell tumors in childhood is considerably greater than recorded. Nevertheless, such tumors account for only 10 per cent of ovarian neoplasms in children[37-39] and an even smaller percentage of cases of sexual precocity. Wilkins[1] encountered only one instance in 20 years of active endocrine practice and Jolly[7] one case in his material collected from various London clinics, which included 31 patients with sexual precocity and early adolescence. In the present series there were but 2 ovarian neoplasms associated with sexual precocity, compared to 49 cases of constitutional precocity.

The symptoms and clinical signs produced by feminizing ovarian tumors are well

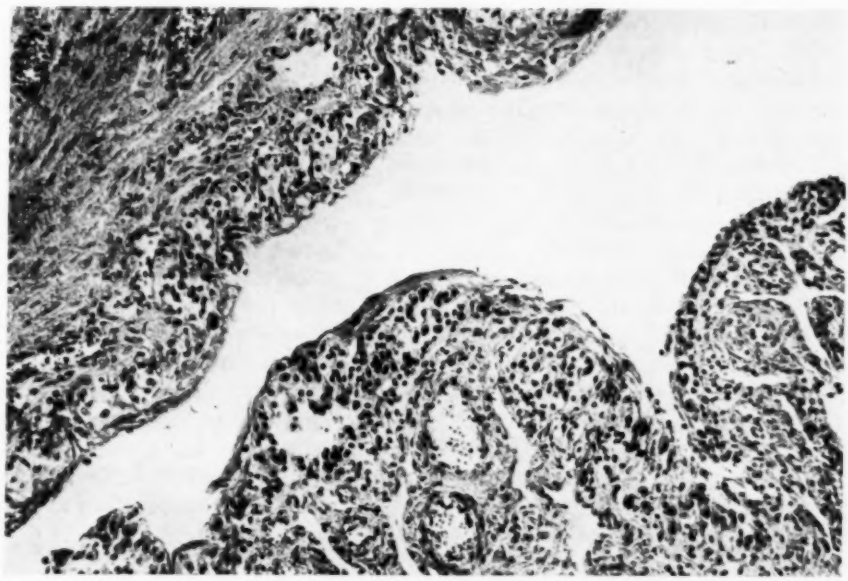


**Fig. 6.** Case 3. Microscopic section of cyst wall. (×100; reduced ¼.)

known.[1, 4, 7, 37, 40-42] Our 2 cases illustrate some of the variations encountered in clinical practice which are worth discussing because they indicate the need for a more critical attitude toward certain constantly repeated statements in the literature on this subject. Almost all authors, for example, emphasize that the rare granulosa cell tumor can be distinguished from the much commoner instance of idiopathic sexual precocity by three criteria: (1) the invariable presence of a palpable abdominal tumor by the time the genitals and breasts are affected; (2) a considerably elevated excretion of estrogens in the urine; and (3) the absence of urinary gonadotropins.

Case 1 demonstrates that a feminizing ovarian tumor is not always palpable early in the course of the disease. This patient was examined under anesthesia by a gynecologist several weeks after the onset of a vaginal discharge, and no tumor was found. Six months later, by which time considerable breast development and a growth spurt had occurred, she was seen by a competent pediatrician who failed to find a tumor by abdominal or rectal examination. It was this fact which delayed referral to our hospital until 1 year later, 4 days after the same physician had discovered a painful lower abdominal mass. In some respects Case 1 resembles that of the leutoma reported by Seckel[14] and emphasizes the need for repeated examinations to exclude the possibility of an ovarian tumor as the cause of sexual precocity. A review of the literature also suggests that occasionally massive ascites may prevent palpation of even a large ovarian tumor, as in the early case of Kussmaul[8] and in the more recent one of Fanconi.[19]

Both Case 1 and Case 2 further show that feminizing tumors are not always characterized by the excretion of large quantities of estrogens in the urine. In Case 1 total urinary estrogens were at a normal level for adolescence. In Case 2 the excretion of estriol was less than that normally measured at mid-cycle or during the luteal phase of the adult menstrual cycle. The initial level was elevated for this subject, however, and progressively fell during the postoperative and immediate follow-up periods. This finding is not particularly remarkable; an adolescent or only slightly higher level of estrogen excretion has been reported with several other granulosa cell tumors in children.[1, 19, 25, 28, 43, 44] It is not definitely known whether this is related to the histology of the tumor, its size, or a phasic secretory activity, in turn dependent upon blood supply.[32] It is well established that cyclic bleeding can occur in the presence of a tumor and closely mimic normal menstruation. At such times production of estrogen by the neoplasm presumably falls and an assay of urine for estrogens might prove to be lower than before or afterward.

Hertig and collaborators[34, 35] believe that the theca cells, not the granulosa cells, are the source of hormones produced by both the normal follicle and follicular tumors. The number of theca cells present in a particular tumor might determine its hormonal activity. Against the premise on which this theory is based, however, and therefore of seeming importance to ovarian histochemistry, is the fact that no theca cells were detected in sections of the first granulosa cell tumor but the tumor cells contained an abundance of fluorescent and birefringent crystals, thought to be specific for the theca cell.[45] This finding, illustrated in Fig. 7, suggests that in ovarian as in adrenal tumors one cannot accurately correlate histology and histochemical findings with specific hormone secretion by the neoplastic cells.

A third finding most authors consider of great diagnostic value is the presence of gonadotropins in the urine of patients with true sexual precocity and their absence in cases of granulosa cell tumor. In our experience less than half of the patients with constitutional precocity, when first examined, are found to excrete gonadotropins detectable by the rather crude bioassay methods presently available. Case 2 demonstrated the completely opposite finding. This patient's neoplasm met all the histologic and clinical criteria of a feminizing granulosa cell tumor, and yet she excreted gonadotropins at a nor-

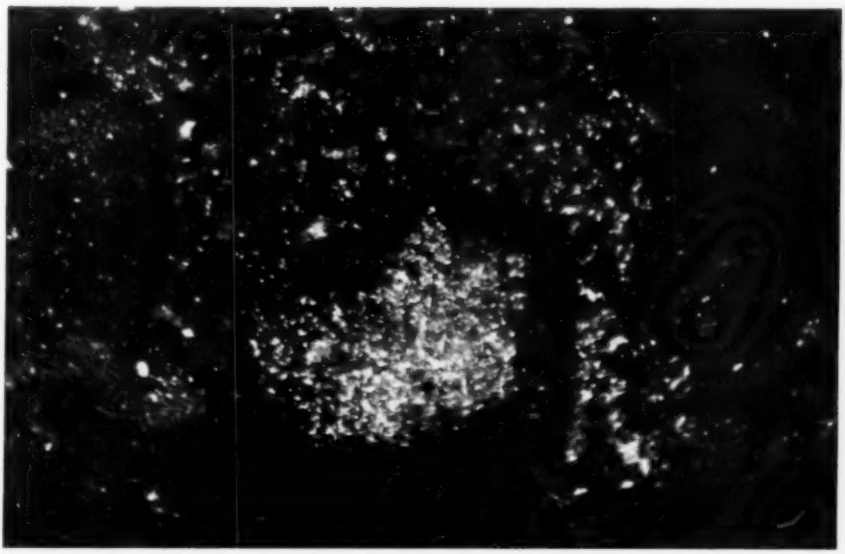


**Fig. 7.** Case 1. Granulosa cell tumor. Fluorescent and birefringent crystals (white areas) remaining after acetone leach. Section is from the same area of tumor as in Fig. 2. Crystals were subsequently shown by color reactions to be sterols. Method of McKay, Robinson, and Hertig.[45] (×120; reduced ¼.)

mal adult level. Although unexpected, this finding is not unique. Pedowitz,[15] reviewing 62 cases of feminizing granulosa cell tumors in children collected from the literature, found among them 12 assays for gonadotropins; in 3 of these an adult excretory level was detected. He was unable to explain this, although noting that very large quantities may be excreted in cases of chorionepithelioma or in teratomas and in dysgerminomas containing choriomatous tissue. It is possible that in Case 2 the moderate estrogen production by the tumor stimulated pituitary secretion of gonadotropins, whereas in the case of tumors producing large amounts of estrogen, suppression of the pituitary results. In support of this theory is the observation made by Paschkis, Rakoff, and Cantarow[46] that certain granulosa cell tumors in adults are accompanied by few manifestations of excess hormone secretion, and these patients may excrete high levels of urinary gonadotropins.

Cases 1 and 2 illustrate quite well that it may occasionally be difficult early in the disease to distinguish a feminizing ovarian tumor from idiopathic sexual precocity by physical examination or laboratory tests. Both demonstrate that sooner or later a tumor, if present, should become palpable, but this may demand repeated physical examinations over a long period of time. In the past the diagnostic problem this presents has been viewed rather complacently by most clinicians, who have thought it preferable to wait for a tumor to appear than to submit a child to an unnecessary exploratory laparotomy.

One reason for a conservative attitude, of course, is the rarity of feminizing tumors in children, but even more important is the widespread belief that granulosa cell tumors in children are almost always benign. This concept seems to stem from the early reviews of Seckel,[6, 13] who recorded only 2 deaths in 31 proved cases of granulosa cell tumor. Pedowitz[15] listed 8 deaths in 49 cases, a fatality rate of 16 per cent, but only 10 of the 41 living patients he surveyed had been followed for 5 years. A 5 year follow-up by

the Ovarian Tumor Registry[47] of 96 cases of granulosa cell tumor, including 6 in children under the age of 10 years, revealed a 21 per cent mortality rate unrelated to age. There is reason to believe that even this figure is misleading, for late recurrences of feminizing tumors are frequent.[48, 49] In his excellent review Diddle[50] cited a case of granulosa cell tumor removed from an 8 year old child which recurred 25 years later.

Since the ultimate prognosis appears to be correlated with the extent of the tumor at initial operation[47] it would appear wise to view the diagnostic problem somewhat less conservatively. Examination under anesthesia is indicated if any adnexal abnormality is detected by bimanual palpation in a sexually precocious girl. It would appear wiser to advise laparotomy, when truly in doubt, than to conclude that slight enlargement of the ovary means constitutional precocity because ovarian tumors are so rare. Failure to palpate a mass obviously does not exclude the presence of a tumor. Repeated physical examinations may be required over an extended period of time.

Examination under anesthesia was not performed on either of the first 2 patients because a mass was evident by the time of referral to this hospital. The diagnosis of feminizing tumor of the ovary was considered well established in Case 3 prior to operation for the same reason, but here the mass proved to be a theca-lutein cyst. It is difficult to decide whether this represents constitutional precocity complicated by an ovarian cyst, in which event the detection of a tumor was misleading, rather than diagnostic, or an autonomous cyst producing precocity. Many authors[1, 7, 15, 31, 38] believe that ovarian cysts are always secondary.

There are several reasons for this belief. In the first place, ovarian cysts are fairly common in childhood, accounting for 25 to 35 per cent of ovarian tumors at this age,[38, 51, 52] but very few are associated with sexual precocity. Even the large cysts which occur in the newborn period and cause mechanical difficulties are neither autonomous nor feminizing.[53-66] They appear to be variants of the small ovarian cysts demonstrable in most young infants coming to autopsy,[67] and are considered physiologic.[68, 69] But perhaps the most cogent reason for doubting that ovarian cysts function independently is the frequency of cysts in girls with constitutional precocity who are submitted to surgical exploration. Novak reported 4 such cases, in all of which the precocity progressed.

Nevertheless, a careful review of the published cases of ovarian cysts associated with precocity leads one to believe that not all were secondary to true precocity, even though as individual case reports they have been rather summarily dismissed as such, often by the authors themselves. Many of the cases were followed or described too briefly to judge whether sexual precocity ceased or progressed after operation.[7, 38, 72, 75, 80] In 8 instances the precocity definitely progressed.[5, 70, 73, 78] This course is not surprising in the patient described by Mason[78] who was not operated on until the age of 8 years, at which time the bone age was advanced and normal adolescent development would be considered imminent. It may or may not be significant that the precocity progressed in all 4 cases reported by Novak[5] which were treated by simple needling of the cysts and biopsy.

There remain 5 cases[18, 71, 74, 76, 77] and possibly a sixth[79] in which a rather remarkable regression of sexual precocity followed ovariectomy and which persisted from 1½ to 4 years. In 2 of the 5 cases[74, 77] adolescent development recurred at an age appropriate for the maturational level of the child, which was already considerably advanced prior to operation. No more than this is achieved by removal of an autonomous, functional granulosa cell tumor from a child of the same physiologic age, as seen in Case 1 of our series. In Mengert's[71] patient not only had the signs of precocity disappeared for 3 years at the time of his report, but estrogen excretion had returned to a prepuberal level. The same was true of Kimmel's[76] patient followed for 1½ years, and the child observed by Tweedie[18] for 15 months. All these

cases showed similar findings at operation: usually one, but occasionally several, cysts of one ovary, the opposite ovary remaining small and grossly normal for a prepuberal child.

There are at least two features common to these 5 cases which seem to set them apart from the others and to cast doubt on the belief that all were instances of constitutional precocity: the gross immaturity of the remaining ovary and the prolonged regression of the signs and symptoms of sexual precocity following removal of the other, diseased one, and the fact that the extent and duration of the remission depended upon the maturational age of the child at the time of operation. Both features resemble the usual clinical course following removal of a granulosa cell tumor, rather than progressive constitutional precocity. Tweedie[18] believed that the static nature of the cyst he observed over a period of weeks prior to operation gave further support to the concept of an autonomous ovarian cyst as a rare but distinctive cause of sexual precocity. Selye,[81] who arrived at a similar viewpoint from study of the older literature, believed that the functional cyst should be differentiated from "small cystic degeneration" of the ovary, and that the latter should be treated by needling rather than by ovariectomy.

Case 3 may represent a further example of the autonomous feminizing cyst of the ovary. Here both the right ovary, which was biopsied, and the remnant of the left ovary, removed with the cyst, were found to contain only immature primordial follicles. The cyst itself proved to be unusual, not only in regard to fibrosis of the capsule but to the anomalous origin from the hilum of the ovary. During the brief follow-up period possible since operation, vaginal bleeding has ceased, breast development has regressed, and no further advance in bone age has occurred.

One should of course be very reluctant to remove an ovary from a child except for pressing reasons. In Case 3 there was no choice, because of the size of the cyst. This unfortunate fact nevertheless will afford an unusual opportunity to study the life history of the child's disease. If she did have a functionally autonmous cyst of the ovary, it was removed at a young enough age to permit complete regression of secondary sexual development. If during the subsequent follow-up period it becomes apparent that the diagnosis is constitutional precocity, to which the cyst was secondary, Case 3 will nevertheless prove instructive as a further example of the occasional difficulty encountered in attempting to distinguish true sexual precocity from pseudoprecocity in the female.

## SUMMARY

Two cases of granulosa cell tumor and one of theca-lutein cyst of the ovary associated with sexual precocity are reported, which demonstrate the difficulty in distinguishing pseudoprecocity from true precocity in the female by the three criteria usually employed: the presence of a mass palpable bimanually, the excretion of large amounts of estrogens, and the absence of urinary gonadotropins when the precocity is produced by a granulosa cell tumor. One of the tumors completely lacked theca cells but contained abundant crystals usually thought to represent the source of estrogens. This finding casts doubt on the belief that the theca cells alone are responsible for estrogen production in ovarian tumors. A review of the literature reveals that granulosa cell tumors in children are less benign than is commonly believed.

An analysis of the published cases of ovarian cysts associated with precocity suggests that not all are variants of true sexual precocity. The theca-lutein cyst reported is a possible example of an autonomous functional cyst, resembling five or six others described in the past.

## REFERENCES

1. Wilkins, L.: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence, ed. 2, Springfield, Ill., 1957, Charles C Thomas, Publisher, p. 189.
2. Talbot, N. B., Sobel, E. H., McArthur, J. W., and Crawford, J. D.: Functional Endocrinology. Cambridge, Mass., 1952, Harvard University Press, chap. V, p. 335.
3. Bongiovanni, A. M., and Eberlein, W. R.:

Pre-adolescent Gynecomastia, Pennsylvania M. J. **60:** 66-70, 1957.
4. Lenz, J.: Vorzeitige Menstruation, Geschlechtsreife und Entwicklung. (Menstruatio, Pubertas et Evolutio praecox) Mit besonderer Berücksichtigung der Skelettentwicklung, Arch. Gynäk. **99:** 67-144, 1913.
5. Novak, E.: Constitutional Type of Female Precocious Puberty With a Report of 9 Cases, Am. J. Obst. & Gynec. **47:** 20-42, 1944.
6. Seckel, H. P. G.: Precocious Sexual Development in Children. M. Clin. North America **30:** 183-209, 1946.
7. Jolly, H.: Sexual Precocity, Springfield, Ill., 1955, Charles C Thomas, Publisher, p. 26.
8. Kussmaul, A.: Über geschlechtliche Frühreife, Würzb. med. Ztschr. **3:** 321-360, 1862.
9. Albert, A.: Procedure for Routine Clinical Determination of Urinary Gonadotropin, Proc. Staff Meet. Mayo Clin. **30:** 552-555, 1955.
10. Eberlein, W. R., Bongiovanni, A. M., and Francis, C. M.: A Simplified Method for the Routine Measurement of Urinary Estriol, J. Clin. Endocrinol. **18:** 1274-1284, 1958.
11. Eberlein, W. R., and Bongiovanni, A. M.: A Paper Chromatographic Method for the Measurement of Pregnanediol in Urine, J. Clin. Endocrinol. **18:** 300-308, 1958.
12. Bongiovanni, A. M., and Eberlein, W. R.: A Critical Analysis of Methods for the Measurement of Pregnane-3α, 17α, 20α-triol in Human Urine, Analyt. Chem. **30:** 388-392, 1958.
13. Seckel, H. P. G., Scott, W. W., and Benditt, E. P.: Six Examples of Precocious Sexual Development. I. Studies in Diagnosis and Pathogenesis, Am. J. Dis. Child. **78:** 484-515, 1949.
14. Seckel, H. P. G., and Plotz, E. J.: Sexual Precocity Due to Estrogen- and Progesterone-Producing Organoid Luteoma of the Ovary Ztschr. Kinderh. **76:** 593-608, 1955.
15. Pedowitz, P., Felmus, L. B., and Mackles, A.: Precocious Pseudopuberty Due to Ovarian Tumors, Obst. & Gynec. Surv. **10:** 633-653, 1955.
16. Clifford, W. S.: Precocious Puberty; Report of a Case in a Female Infant, J. M. A. Georgia **44:** 231-232, 1955.
17. Butt, J. A.: Ovarian Tumors in Children, Am. J. Obst. & Gynec. **69:** 833-837, 1955.
18. Tweedie, F. J.: Precocious Pseudopuberty of Ovarian Origin With 2 Case Reports, Am. J. Obst. & Gynec. **75:** 964-969, 1958.
19. Fanconi, G.: Über Störungen der Pubertät, Deutsche med. Wchnschr. **80:** 337-343, 1955.
20. Hamuy, J., and Riera, J.: Pubertad precox con tumor hiperfuncionante de ovario, Arch. pediat., Uruguay **26:** 468-471, 1955.
21. Jacinto Simoes, J.: Puberdade precoce por tumor de celulas granulosas do ovário. Rev. ibér. endocrinol. **2:** 355-376, 1955.
22. Lelong, M., Joseph, R., Vialatte, J., Le Tan Vinh, and Canlorbe, P.: Tumeur de la granulosa chez une fille de 3 ans ½, Arch. franç. pédiat. **12:** 708-715, 1955.
23. Ofstad, E.: Pubertas praecox ved granulosacelletumor i ovarium, Tidsskr. norske laegefor. **76:** 193-213, 1956.
24. Picanol, J.: Pubertad precoz por tumor de células de la granulosa del ovario, Rev. espan. pediat. **12:** 309-318, 1956.
25. Pattee, C. J., Tweedie, F. J., and Geddes, A. K.: Sexual Precocity; Report of an Ovarian Tumour in a Girl of 19 Months, Canad. M. A. J. **76:** 573-576, 1957.
26. Gürson, C. T., Gorbon, B. R., Pars, D. B., and Bedir, Ö.: Tumeur à cellules granulosa chez un enfant de 15 mois, Helvet. paediat. acta **12:** 548-552, 1957.
27. de Queiroz, A. P., and Freitas, A. L.: Tumor de células da granulosa do ovário, com puberdade precoce, em paciente de 6 anos, An. brazil. ginec. **43:** 1-10, 1957.
28. Degoy, A., Gay, R., Planel, M., and Guilhem, A.: Puberté précoce par tumeur maligne de l'ovaire chez une fillette de sept ans, Arch. franç. pediat. **15:** 558-563, 1958.
29. Destro, F.: Considerazioni di uncaso di neoplasia maligna bilaterale dell'ovaia, Riv. ostet., Milano **40:** 295-304, 1958.
30. Moll, H.: Granulosazelltumor bei einem Säugling, Arch. Kinderh.**157:** 255-258, 1958.
31. Novak, E.: Gynecological and Obstetrical Pathology, ed. 4, Philadelphia, 1958, W. B. Saunders Company, pp. 414-434.
32. Traut, H. F., Kuder, A., and Cadden, J. F.: A Study of the Reticulum and of Luteinization in Granulosa and Theca Cell Tumors of the Ovary, Am. J. Obst. & Gynec. **38:** 798-813, 1939.
33. Meyer, R.: The Pathology of Some Special Ovarian Tumors and Their Relation to Sex Characteristics, Am. J. Obst. & Gynec. **22:** 697-713, 1931.
34. Schultheiss, H. R.: Theka- und Granulosazelltumor, Gynaecologia **146:** 382-399, 1958.
35. Hertig, A. T., and Mansell, H.: Feminizing Mesenchymal Tumors, in Anderson, W. A. D., editor: Pathology, ed. 3, St. Louis, 1957, The C. V. Mosby Company, chap. 39, p. 1076.
36. Ziegler, E. E.: Bilateral Ovarian Carcinoma in a Thirty Week Fetus, Arch. Path. **40:** 279-282, 1945.
37. Gross, R. E.: The Surgery of Infancy and Childhood, Philadelphia, 1953, W. B. Saunders Company, p. 499.
38. Costin, M. E., Jr., and Kennedy, R. L. J.: Ovarian Tumors in Infants and Children, Am. J. Dis. Child. **76:** 127-153, 1948.
39. Steck, A. M.: Les tumeurs ovariennes chez l'enfant, Helvet. paediat. acta **9:** 69-88, 1954.
40. Geist, S. H.: Ovarian Tumours in Children, New York, 1942, Paul B. Hoeber, Inc., chap. XI, pp. 449-451.
41. Israel, S. L., and Mutch, J. C.: Endocrinologic Effects of Certain Ovarian Tumors, Surg. Gynec. & Obst. **105:** 166-176, 1957.
42. Lull, C. B.: Pubertas Praecox Due to Ovarian Tumors, Am. J. Obst. & Gynec. **41:** 445-454, 1941.

43. Stabler, F., and Thomson, J. G.: Granulosa-Cell Tumor With Precocious Sexual Development in a Child Aged 6, J. Obst. & Gynaec. Brit. Emp. **47:** 199-205, 1940.
44. Rinvik, R.: Granulosa Cell Tumour in a Case of Precocious Puberty, Acta obst. et gynec. scandinav. **32:** 222-228, 1953.
45. McKay, D. G., Robinson, D., and Hertig, A. T.: Histochemical Observations on Granulosa-Cell Tumors, Thecomas and Fibromas of the Ovary, Am. J. Obst. & Gynec. **58:** 625-639, 1949.
46. Paschkis, K. E., Rakoff, A. E., and Cantarow, A.: Clinical Endocrinology, ed. 2, New York, 1958, Paul B. Hoeber, Inc., p. 489.
47. Busby, T., and Anderson, G. W.: Feminizing Mesechymomas of the Ovary, Am. J. Obst. & Gynec. **68:** 1391-1420, 1954.
48. Engle, R. B.: Roentgen Therapy of Granulosa-Cell Carcinoma of Ovary, Am. J. Roentgenol. **80:** 793-798, 1958.
49. Kottmeier, H. L.: Carcinoma of the Female Genitalia, Baltimore, 1953, Williams & Wilkins Company, chap. V, p. 186.
50. Diddle, A. W.: Granulosa- and Theca-Cell Ovarian Tumors: Prognosis, Cancer **5:** 215-228, 1952.
51. Bray, P. T., and Thomas, L. P.: Two Cases of Ovarian Cysts in Childhood, Brit. M. J. **1:** 82, 1954.
52. Witzberger, C. M., and Agerty, H. A.: Ovarian Tumors in Infancy and Childhood; With Report of a Case and a Review of the Literature, Arch. Pediat. **54:** 339-348, 1937.
53. Gerlach, W.: Über eine sehr grosse Follikelcyste des Ovars beim Neugeborenen, Zentralbl. f. Gynäk. **62:** 1206-1212, 1938.
54. Tyson, W. T.: Keegan, J. M., and Gold, D.: Large Ovarian Cyst in a Newborn Infant, A. M. A. J. Dis. Child. **94:** 320-321, 1957.
55. Brune, W. H., Pulaski, E. J., and Shuey, H. E.: Giant Ovarian Cyst; Report of a Case in a Premature Infant, New England J. Med. **257:** 876-878, 1957.
56. Bulfamonte, J. C.: Large Ovarian Cyst in Newborn Child, Am. J. Surg. **55:** 175-176, 1942.
57. Downes, W. A.: Tumors of Ovary in Children, J. A. M. A. **76:** 443-445, 1921.
58. Finola, G. C., Quitangon, L., and Pontius, G. V.: Large Ovarian Cyst in a Newborn Infant, Am. J. Obst. & Gynec. **73:** 690-692, 1957.
59. Graves, G. Y., McIlvoy, D. B., Jr., and Hudson, G. W.: Ovarian Cyst in Premature Infant, A. M. A. Am. J. Dis. Child. **81:** 256-258, 1951.
60. Kunstadter, R. H., Schultz, A., and Strauss, A. A.: Large Ovarian Cyst in Newborn Infant, Am. J. Dis. Child. **80:** 993-998, 1950.
61. Parry, N. G.: Large Ovarian Cyst in Newborn Infant, Quart. Bull. Northwestern Univ. M. School **29:** 396-399, 1955.
62. Neikirk, W. I., and Hudson, H. W., Jr.: Giant Ovarian Cyst in Newborn Infant, New England J. Med. **239:** 468-469, 1948.
63. Parkinson, T. J.: Ovarian Cyst With Torsion and Rupture in an Infant, J. Obst. & Gynaec. Brit. Emp. **65:** 84-86, 1958.
64. Smith, R. C.: Simple Ovarian Cyst in Newborn Infant; Case Report, Pediatrics **14:** 232-234, 1954.
65. Tietz, K. G., and Davis, J. B.: Ruptured Ovarian Cyst in a Newborn Infant, J. PEDIAT. **51:** 564-565, 1957.
66. Watson, E. A., and Henderson, A. T.: Giant Ovarian Cyst of Newborn; Report of a Case, Nebraska M. J. **40:** 397-398, 1955.
67. Polhemus, D. W.: Ovarian Maturation and Cyst Formation in Children, Pediatrics **11:** 588-594, 1953.
68. Colberg, E. J.: The Development of Follicular Cysts in the Prepubertal Period and Their Relation to Epithelial Tumors of the Ovary, Papers Mayo Found. & Med. School (1921-1922) **2:** 233-234, 1923.
69. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, 1928, W. B. Saunders Company, p. 624.
70. Meurer: Bericht. Follikelcyst des rechten Ovariums. Zentralbl. Gynäk. **29:** 1302, 1905.
71. Mengert, W. F.: Precocious Puberty Due to Ovarian Cyst in 5-Year-Old Female, Am. J. Obst. & Gynec. **37:** 485-488, 1939.
72. Fischer, H. S.: Precocious Menstruation, Am. J. Obst. & Gynec. **39:** 525-527, 1940.
73. Sternberg, W. H., and Joseph, V.: Osteodystrophia Fibrosa Combined With Precocious Puberty and Exophthalmic Goiter; Pathologic Report of Case, Am. J. Dis. Child. **63:** 748-783, 1942.
74. Goldberg, M. B., Maxwell, A. F., and Smith, P. M.: Three Unusual Endocrinopathies With Associated Ovarian Pathology: I. Ovarian Agenesis. II. Precocious Puberty. III. Virilism, J. Clin. Endocrinol. **7:** 11-29, 1947.
75. Hain, A. M.: The Constitutional Type of Precocious Puberty, J. Clin. Endocrinol. **7:** 171-185, 1947.
76. Kimmel, G. C.: Sexual Precocity and Accelerated Growth in a Child With Follicular Cyst of Ovary, J. PEDIAT. **30:** 686-690, 1947.
77. Hoge, R. H.: Precocious Puberty in Girls, Am. J. Obst. & Gynec. **57:** 388-390, 1949.
78. Mason, L. W.: Precocious Puberty, J. PEDIAT. **34:** 730-740, 1949.
79. Pray, L. G.: Sexual Precocity in Females; Report of Two Cases, With Arrest of Precocity in the McCune-Albright Syndrome After Removal of a Cystic Ovary, Pediatrics **8:** 684-692, 1951.
80. Case Records of the Massachusetts General Hospital. Case 43461, New England J. Med. **257:** 987-992, 1957.
81. Selye, H.: Encyclopaedia of Endocrinology, Montreal, Canada, 1946, Richardson, Bond and Wright, sect. IV, vol. VII, pp. 35-38, 46-50, 65-68.

# *News and notes*

**The American Association of Poison Control Centers** will hold its third annual meeting at the Palmer House, Chicago, October 18, with a morning business meeting and an afternoon scientific session. Fifteen- to 20-minute papers dealing, for the most part, with poisoning in children, but also in adults, will be given with a 5-minute discussion following each. For further information contact Harry C. Shirkey, M.D., The Children's Hospital, 712 South 30th Street, Birmingham, Ala.

For several years scientists interested in basic problems of congenital malformations have held informal conferences in which questions of common interest were discussed. Anatomists, biochemists, embryologists, geneticists, obstetricians, pathologists, pediatricians, plastic surgeons, and others attended these conferences which were in part supported by the Association for the Aid of Crippled Children, New York, and the Human Embryology and Development Study Section of the National Institutes of Health. With the increased interest in recent years in this area, it was felt that there was a need for a society to hold regular meetings in which investigations concerned with the etiology and morphogenesis of congenital malformations could be presented and discussed.

Following the fourth teratology conference, which was held at the Memorial Sloan-Kettering Cancer Center in New York City and attended by 76 scientists from Canada, England, France, Germany, and the United States, **The Teratology Society** was formed for the purposes outlined above. The following officers were elected: President: Josef Warkany, M.D., Cincinnati, O.; President-Elect: James G. Wilson, Ph.D., Gainesville, Fla.; Secretary-Treasurer: Marjorie M. Nelson, Ph.D., San Francisco, Calif.; Recorder: Sidney Q. Cohlan, M.D., New York, N. Y.; Council: F. Clarke Fraser, Ph.D., M.D., Montreal, Canada, David L. Gunberg, Ph.D., Portland, Ore., and M. Lois Murphy, M.D., New York, N. Y. The National Foundation assisted in the formation of the Society with advice and financial aid. Inquiries about The Teratology Society should be directed to Dr. Marjorie M. Nelson, Department of Anatomy, School of Medicine, University of California, San Francisco 22, Calif.

The **American Academy of Pediatrics** announces the first two of the Postgraduate Courses it is sponsoring from July, 1960, to July, 1961. The first course, **Pediatric Endocrinology and Genetics,** will be given by the State University of New York at the Upstate Medical Center (Syracuse) under the direction of Dr. Lytt Gardner, November 9 to 11. The second course, **Psychological Aspects of Pediatrics,** will be given at the Yale University School of Medicine under the direction of Dr. Milton Senn, November 28 to 30.

Tuition for these courses is $40 for Academy Fellows and $60 for nonmembers. Registration is limited. For registration information write to the American Academy of Pediatrics, 1801 Hinman Avenue, Evanston, Illinois.

**The Chicago Pediatric Society,** at its annual meeting elected the following as officers for the year 1960-1961: President, Dr. Matthew M. Steiner; Vice-President, Dr. James A. Conner; Secretary, Dr. Harry L. Faulkner; Treasurer, Dr. I. Pat Bronstein; Editor, Dr. Howard S. Traisman, and Executive Committee, Drs. Ralph H. Kunstadter, Noel G. Shaw, and Raymond F. Grissom.

**The American Board of Psychiatry and Neurology, Inc.,** and its Committee on Certification in Child Psychiatry, announce the certification of the following as Diplomates in child psychiatry:

James Milton Bell, M.D., Berkshire Farm for Boys, Canaan, New York

Norman Ralph Bernstein, M.D., 906 Warren Building, 275 Charles Street, Boston 14, Massachusetts

A. Ferdinand Bonan, M.D., 133 South 36th Street, Philadelphia 4, Pennsylvania

John Armistead Boston, Jr., M.D., 314 West 17th Street, Austin, Texas

Jack Emile Chappuis, M.D., 1737 Prytania Street, New Orleans, Louisiana

Walter F. Char, M.D., 3432 North Broad Street, Philadelphia 40, Pennsylvania

Richard Lawrence Cohen, M.D., 1030 South Concord Road, West Chester, Pennsylvania

Theodore Benjamin Cohen, M.D., Presidential Apartments, D 104, Philadelphia 31, Pennsylvania

Albert Victor Cutter, M.D., 57 Linwood Avenue, Buffalo 9, New York

Carlo P. DeAntonio, M.D., 4835 Van Nuys Boulevard, Sherman Oaks, California

Chester R. Dietz, M.D., 2013 Baynard Boulevard, Wilmington 2, Delware

Leon Eisenberg, M.D., Johns Hopkins Hospital, Baltimore 5, Maryland

Aaron H. Esman, M.D., 4 East 95th Street, New York 28, New York

Stuart McIntyre Finch, M.D., Children's Psychiatric Hospital, Ann Arbor, Michigan

Joseph Fischhoff, M.D., 19304 Wyoming, Detroit 21, Michigan

Barbara Fish, M.D., 106 East 85th Street, New York 28, New York

Robert B. Forman, M.D., 3617 West Sixth, North Office, Topeka, Kansas

Harry George Gianakon, M.D., 110 North 48th Street, Philadelphia 39, Pennsylvania

Alfred J. Gianascol, M.D., Langley Porter Neuropsychiatric Institute, First and Parnassus Avenues, San Francisco 22, California

Arthur E. Gillman, M.D., 7 West 96th Street, New York, New York

Joseph Martin Green, M.D., University Hospitals, 1300 University Avenue, Madison 6, Wisconsin

Robert L. Greenlee, M.D., 1110 W. Washington, Fort Wayne, Indiana

Harold Joseph Harris, M.D., North Carolina Memorial Hospital, Chapel Hill, North Carolina

Melvin S. Heller, M.D., Presidential D104, City Line Avenue, Philadelphia 31, Pennsylvania

Richmond Holder, M.D., 274 Commonwealth Avenue, Boston 16, Massachusetts

Leonard Hollander, M.D., Albert Einstein College of Medicine, Eastchester Road and Morris Park Avenue, Bronx 61, New York

Albert S. Hotkins, M.D., 109-23 71st Road, Forest Hills 75, New York

Doris M. Hunter, M.D., 3811 O'Hara Street, Pittsburgh 13, Pennsylvania

Jean D. Jameson, M.D., 138 East 71st Street, New York City 21, New York

Jacob Philip Kahn, M.D., 3261 Clay Street, San Francisco, California

Harris Elliott Karowe, M.D., 835 Union Street, Schenectaday 8, New York

Michael Theodore Khlentzos, M.D., 2200 Hayes, San Francisco 17, California

Elizabeth Kleinberger, M.D., 1060 Park Avenue, New York 28, New York

LeRoy F. Kurlander, M.D., Child Guidance Clinic of San Diego, 8001 Frost Street, San Diego 11, California

James Joseph Lawton, Jr., M.D., University of Minnesota Medical School, Minneapolis 14, Minnesota

Edward Mortimer Litin, M.D., Mayo Clinic, 200 First Street, SW, Rochester, Minnesota

Irwin M. Marcus, M.D., 3619 Prytania, New Orleans, Louisiana

John Tarlton Morrow, Jr., M.D., The Menninger Foundation, Children's Service, 2221 West Sixth Avenue, Topeka, Kansas

Byron Leon Nestor, M.D., 2522 Dana Street, Berkeley, California

Joseph D. Noshpitz, M.D., Clinical Center, National Institute of Mental Health, Bethesda 14, Maryland

Morris Parmet, M.D., Hunterdon Medical Center, Flemington, New Jersey

Robert C. Prall, M.D., Henry Avenue and Abbottsford Road, Philadelphia 29, Pennsylvania

Francis T. Rafferty, M.D., 156 Westminster Avenue, Salt Lake City, Utah

John Belvin Reinhart, M.D., 125 DeSoto Street, Pittsburgh 13, Pennsylvania

Bertram Aaron Ruttenberg, M.D., 101 Wooded Lane, Villanova, Pennsylvania

Calvin Frederick Settlage, M.D., 105 Woodside Road, Ardmore, Pennsylvania

Marvin I. Shapiro, M.D., 201 DeSoto Street, Pittsburgh 13, Pennsylvania

Malcolm Richard Sills, M.D., 20 Institute Road, Worcester, Massachusetts

Archie Aaron Silver, M.D., 460 East 63rd Street, New York 21, New York

James E. Simmons, M.D., Indiana University Medical Center, Indianapolis, Indiana

Meyer Sonis, M.D., 1700 Bainbridge Street, Philadelphia 46, Pennsylvania

James Neil Sussex, M.D., 620 South 20th Street, Birmingham 3, Alabama

Charles R. Swift, M.D., 532 W. State Street, Trenton 8, New Jersey

David S. Thorsen, M.D., 670 Marshall Avenue, St. Paul 4, Minnesota

Jack Vance Wallinga, M.D., 215 Doctors Building, Minneapolis, Minnesota

Richard Storer Ward, M.D., 80 Irving Place, New York 3, New York

Morris Weiss, M.D., 634 Maccabees Building, Detroit 2, Michigan

Virginia Nichols Wilking, M.D., Pediatric Psychiatric Clinic, Columbia-Presbyterian Medical Center, 622 W. 168th Street, New York 32, New York

Henry H. Work, M.D., School of Medicine, Department of Psychiatry, University of California Medical Center, Los Angeles 24, California

Stephen C. Wright, M.D., 1333 South Miami Avenue, Miami, Florida

Thomas S. Wright, M.D., Abington Memorial Hospital, Abington, Pennsylvania

Howard Lee Wylie, M.D., 135 Woodland Drive, Pittsburgh 28, Pennsylvania

**The Second Invitational Conference of the Napa State Hospital** presented by Napa and Sonoma State Hospitals in cooperation with the Ames Company, Inc., will be held October 8, and will be limited to 120 professional persons including psychiatrists, psychologists, biochemists, geneticists, pediatricians, and other interested persons.

Participants include Drs. David Yi-Yung Hsia, Norman Kretchmer, Stanley W. Wright, Willard R. Centerwall, Alfred G. Knudsen, Jon Karlsjon, Carolyn F. Piel, and Robert W. Day.

For further information write to Dr. David Wardell, Chief of Professional Education, Sonoma State Hospital, Eldridge, Calif.